Rapid Screening Methods to Identify Substandard and Falsified Medicines

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Declaration

This thesis contains the original work of the author except where otherwise indicated.

Abstract

Substandard and falsified medicines (SF) are a major global public health problem and occur throughout healthcare systems worldwide. With an average occurrence of 10% of all medicines globally, the specific levels range from ~1% of all medicines in high-income countries (HIC) to as much as 30-40% in low-income countries (LIC) and low-middle income countries (LMIC).

Current detection methods for SF medicines range from 'low tech' approaches, for example, visual appearance, thin layer chromatography (TLC) to those that are technically refined, such as, Raman spectroscopy, Near Infrared (NIR) spectroscopy and Mass Spectrometry (MS). In order to counteract the increasing complexity of anti-counterfeit measures, counterfeiters are finding ever more sophisticated ways to bypass existing screening techniques. Therefore, the aim of this research is to develop a novel rapid screening method to identify SF medicines.

The performance of seven different benchtop instruments has been investigated with respect to their potential to deliver rapid assessment of the identity and to quantify the level of Active Pharmaceutical Ingredient's (API's) present in tablet samples.

The materials used in this research comprised of 29 reference samples and 64 individual group samples from 8 countries, totaling some 867 tablets. Tablets were analysed in both whole and powdered forms.

Mass Spectrometry provided the least complex data, followed by Raman and then Attenuated Total Reflectance Fourier Transform Infrared (ATR FTIR). The mass spectrometer was the least reliable instrument, but provided the greatest sensitivity.

Successful identification of the API was obtained from ATR FTIR and Raman analysis and from Direct Insertion Probe (DIP) Mass Spectrometry.

Quantitative levels of API could be obtained from Ultraviolet (UV) and ATR FTIR measurements, whilst some excipient levels could be determined by Elemental Dispersive X-ray (EDX) spectroscopy. Handheld Raman systems produced some erroneous quantitative information.

The majority of samples examined were within ±10% of the stated level, as per the British Pharmacopeia specifications; however, there was some evidence of substandard medication. Substandard medication was suspected in 2 of the 64 pharmaceutical products assessed.

At the present state of development, the Raman and ATR FTIR equipment could be used in LMIC's for the screening of tablet samples.

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Outputs Resulting from this Research

Peer Reviewed Journal Publications

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Conference Oral Presentations

Armitage, R. (2017) Identification of Counterfeit Medicines – A Truly Global Issue. Health and Life Sciences Conference, De Montfort University, Leicester, March 2017.

Lawson, G., Armitage, R. and Tanna, S. (2012) Identification of Counterfeit Drugs. Invited lecture at Cafe Scientifique, Nanjing, China, November 2012.

Lawson, G., Armitage, R. and Tanna, S. (2012) Identification of counterfeit medicines. 7th Annual Pharmaceutical Anti-Counterfeiting Meeting. 2012, Visiongain Conference Centre, Barbican, London, UK. 26-27 June.

Conference Poster Presentations

Armitage, R., Lawson, G. and Tanna, S. (2017) Counterfeit or just poor quality control? Presented at 8th APS International PharmSci conference, University of Hertfordshire, Hatfield. 5th – 7th September 2017.

Tanna, S., Lawson, G., Armitage, R. and Ogwu, J. (2017) #DMU engage – Helping people in Africa stay clear of fake and substandard medicines #DMU engage and #DMU global trip to Nairobi, 19 June-26 June 2017.

Lawson, G., Ogwu, J., Armitage, R., Alcroft, C. and Tanna, S. (2016) Fast identification of counterfeit medicines – a comparison of two MS methods. Proceedings of the 21st International Mass Spectrometry conference, Toronto, Canada. 20 – 26 August 2016.

Tanna S., Armitage R., Lawson G. (2013) Identification of counterfeit pills - Is rapid instrumental analysis possible? Proceedings of the 24th International Symposium on Pharmaceutical and Biomedical Analysis, 30 June-3 July 2013.

Keeling, R., Lawson, G., Needham, M., Tanna, S., (2011) A Microscopic Approach to a Big Problem… … Analysing Counterfeit Drugs. Presented at the 6th annual Vitae Midlands Hub Regional PhD Poster Competition, Nottingham Trent University, Nottingham, July 2011.

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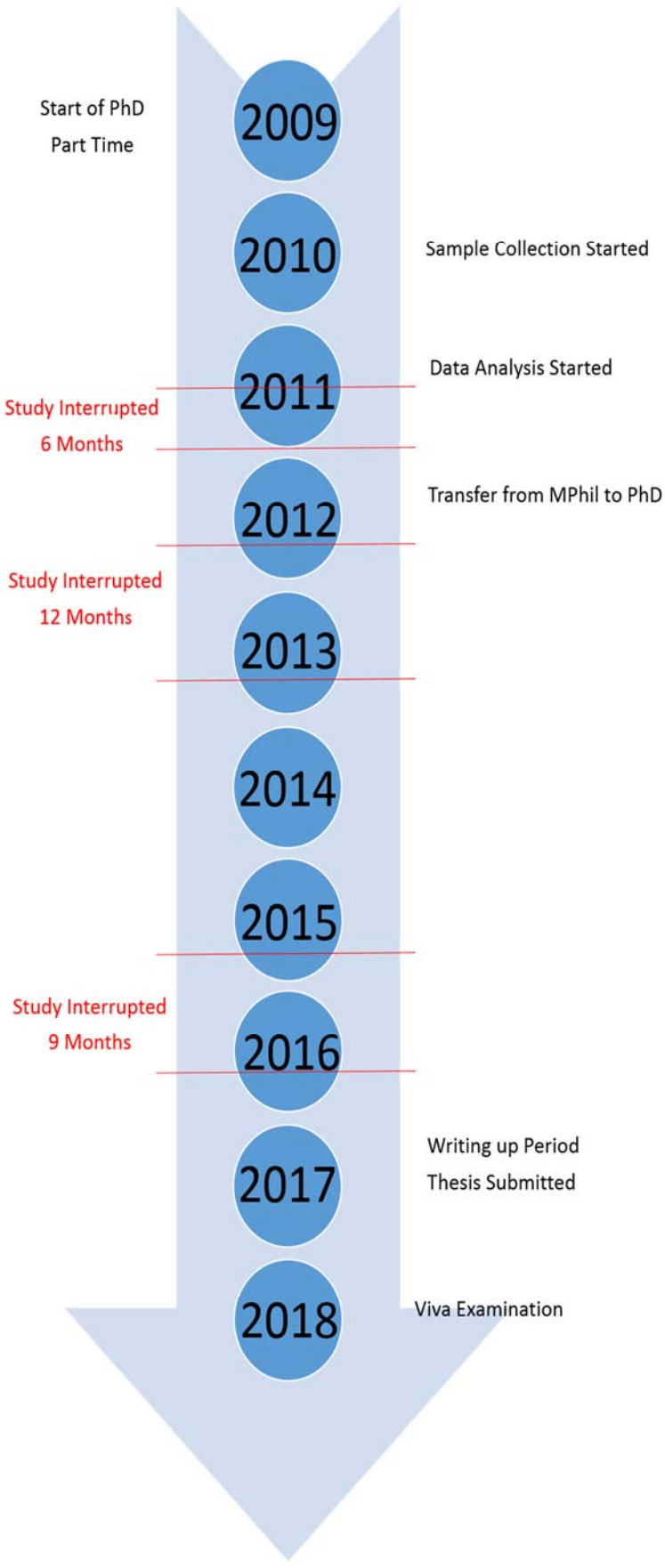
Keeling, R., Lawson, G., Tanna, S., Needham, M., (2010) It's not what it says on the packet… …Counterfeit drugs. Presented at the FIRN Midlands regional student forensic science conference, De Montfort University, Leicester, April 2010.

Conference Proceedings

Lawson, G., Armitage, R., Alhedethe, A. and Tanna, S. (2013) Rapid identification of counterfeit pills by ATR FT/IR analysis of crushed samples. *Journal of Analytical and Bioanalytical Techniques*, 4 (5), p. 50.

Timeline of PhD Study



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Abbreviations

ACT	Artemisin based Combination Therapies
ADR	Adverse Drug Reaction
API	Active Pharmaceutical Ingredient
ASAP	Atmospheric Solid Analysis Probe
ATR	Attenuated Total Reflectance
BP	British Pharmacopeia
BSE	Backscattered Electron
CIS	Counterfeit Incident System
CL	Cathodoluminscence
DIP	Direct Insertion Probe
EAASM	European Alliance for Access to Safe Medicines
EC	European Commission
EDX	Energy Dispersive X-ray Spectroscopy
EI	Electron Impact
EM	Electromagnetic
EMA	European Medicines Agency
EP	European Pharmacopeia
EU	European Union
FDA	Food and Drug Administration
FIA	Federal Investigation Agency
FTIR	Fourier Transform Infrared
GPHF	Global Pharma Health Fund

HIC	High-income countries
HIV/AIDS	Human Immunodeficiency Virus / Acquired Immune Deficiency Syndrome
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl methyl cellulose
IID	Inactive Ingredient Database
IMPACT	International Medical Products Anti-Counterfeiting Taskforce
INTERPOL	International Police
IP	Intellectual Property
IPN	International Policy Network
IR	Infrared
LC MSMS	Liquid Chromatography Tandem Mass Spectrometer
LIC	Lower income countries
LMIC	Lower middle-income countries
MCC	Microcrystalline Cellulose
MHRA	Medicines and Healthcare Products Regulatory Agency
MS	Mass Spectrometry
MSF	Médecins Sans Frontières
NHS	National Health Service
NIR	Near Infrared
NMR	Nuclear Magnetic Resonance
OTC	Over the Counter
PCA	Principal Component Analysis
PEG	Polyethylene glycol
PIC	Punjab Institute of Cardiology

PLS	Partial Least Squares
POM	Prescription Only Medicine
PSI	Pharmaceutical Security Institute
PVP	Polyvinylpyrrolidinone
QC	Quality Control
SE	Secondary Electron
SEM	Scanning Electron Microscopy
SEM/EDX	Scanning Electron Microscopy/ Energy Dispersive X-ray Spectroscopy
SERS	Surface Enhanced Raman Spectroscopy
SF	Substandard and falsified medical products
SP	Sulphadoxine - Pyrimethamine
SSFFC	Substandard, spurious, falsely labelled, falsified and counterfeit medical products
TIC	Total Ion Chromatogram
TLC	Thin Layer Chromatography
UK	United Kingdom
UNODC	United Nations Office on Drugs and Crimes
US	United States
USA	United States of America
UV	Ultraviolet
WADA	World Anti-Doping Agency
WHO	World Health Organisation

Glossary

Atlas Conversion Factor: For any given year, it is the average of a country's exchange rate for the preceding two years, adjusted for the difference between the rate of inflation in the country and international inflation.

Artesunate: A water-soluble hemisuccinate derivative of artemisinin.

Bioavailability: The rate and extent to which the API is absorbed from a pharmaceutical dosage form and becomes available in the general circulation.

Bioequivalence: Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent and their bioavailability after exposure.

Dosage form: The finished formulation of a pharmaceutical product, for example tablet, capsule and suspension.

Generic product: A multisource pharmaceutical product, which is usually manufactured without a licence from the innovator company and marketed after the expiry of a patent.

High Income Countries: Gross National Income (GNI) per capita calculated using World Bank Atlas method of $12,476 or higher per annum.

Innovator product: A pharmaceutical product that was first authorised for marketing based on documentation of quality, safety and efficacy.

Low Income Countries: GNI per capita calculated using World Bank Atlas method of $1,025 or less per annum.

Lower Middle-Income Countries: GNI per capita calculated using World Bank Atlas method of between $1,026 and $4,035 per annum.

Upper Middle-Income Countries: GNI per capita calculated using World Bank Atlas method of between $4,036 and $12,475 per annum.

World Bank Atlas method: In calculating GNI in US Dollars, the Atlas conversion factor is used instead of exchange rates. The Atlas conversion reduces the impact of exchange rate fluctuations.

1 Introduction

This Chapter looks at how substandard and falsified (SF) medicines are a global issue that affects both High Income Countries (HIC) and Low Income Countries (LIC), spanning across both lifesaving and lifestyle medicines. Whilst this issue is more prevalent in LIC and Lower Middle Income Countries (LMIC), it is a growing market worldwide and in part due to the rise in internet pharmacies.

Governments and the pharmaceutical industry are trying to stem the tide of SF medicines, with the creation of new laws, taskforces and the implementation of the Falsified Medicines Directive in the EU. However, before the new laws can be implemented, we need to understand the formulation of medicines and in particular tablets. Chapter 2 discusses the selection of target medicines and the different components in a tablet.

1.1 Background

In January 2012, the city of Lahore in Pakistan experienced what was described by the media as a 'fake medicine crisis'. Over one hundred cardiac patients died during a three-week period, as a direct result of taking substandard/falsified cardiovascular medication (BBC, 2012a). The contaminated medication had been distributed in mid-December by the Government run Punjab Institute of Cardiology (PIC) (BBC, 2012a). It was reported that around forty thousand people were at risk from taking the free medication (Hugman, 2012), in particular the less privileged, as the medication was mainly supplied to them (Riaz, 2012). The affected patients exhibited symptoms similar to dengue fever, these included the rapid depletion of white blood cells and blood platelets, bleeding from the mouth and gastrointestinal tract and strange dark marks on the skin (WHO, 2013). Doctors initially mistook the outbreak for dengue fever (Riaz, 2012); however, there had been reports of zero findings of the dengue mosquito larvae (WHO, 2013) and that the symptoms were limited to patients treated by the PIC (Riaz, 2012). The Federal Investigation Agency (FIA) found that the medicines supplied to patients were manufactured in unhygienic conditions with no adherence to safety regulations (Hao, 2012). Samples of the medication were analysed by the Medicines and Healthcare Products Regulatory Agency (MHRA) in England and found to contain pyrimethamine, an antiparasitic drug used in the treatment of malaria. The presence of this active ingredient was discovered to be toxic to the patients (Riaz, 2012). The MHRA also found that this particular batch of cardiovascular medication had been manufactured at a facility that manufactured both cardiovascular and

antimalarial medication (WHO, 2013). Not only was the drug misidentified, but the packaging also failed to display any dates of manufacture or expiry (BBC, 2012a).

Counterfeiting of commercial products is an age-old practice, which flourishes in many countries and is motivated mainly by the huge profits that can be made (WHO, 1999). The production of substandard and counterfeit medicines is a vast and under reported problem, particularly affecting poorer countries (Cockburn *et al*., 2005; Höllein and Holzgrabe, 2014 and Glass, 2014). High costs and increasing public demand for newer medicines means that high profits can be made from selling counterfeit medicines.

Counterfeit medicines are not restricted to specific drug classes, but instead span a broad spectrum, including lifestyle and lifesaving medicines (WHO, 2010a). Patients that take such medicines as part of their treatment may get no relief from their symptoms and may suffer induced illness or death (Attaran *et al*., 2012).

1.2 Counterfeits become substandard or falsified medicines

Despite the increasing awareness on the dangers of the 'fraudulent drug epidemic' (Lui and Lundin, 2016 and Nayyar *et al*., 2015) there are still issues when assessing the global problem of counterfeit medicines (Fernandez *et al*., 2008). There was no universally accepted definition of counterfeit medicines; this made the exchange of information between countries difficult, as each may have had a conflicting definition (WHO, 2011). The lack of a universal definition led to difficulties in taking effective and strategic actions to solve the issue, (Clift, 2010), and is thought by some sources to be the 'heart of the problem' (Lui and Lundin, 2016 and Gostin *et al*., 2013). To address this problem, the World Health Organisation (WHO) developed the following definition in the 1990's. It was seen as the most elaborate, and had gained international acceptance (Höllein *et al*., 2016).

"A counterfeit medicine is one which is deliberately and fraudulently mislabelled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products, may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging" (WHO, 2011).

However, the term counterfeit is now deemed to be controversial by some parties including the WHO, who removed the term counterfeits from its definition in early 2017. Although the above definition by the WHO remained generally accepted as the worldwide standard (Dégardin *et al*., 2014); concerns that the term counterfeit is largely associated and defined, with respect to intellectual property (IP) legislation, adds complexity to the connotation of the term (Lui and

Lundin, 2016). As a result of pressure from stakeholders and criticism that the enforcement of the IP issue would empower big pharmaceutical companies and hinder the generic industry in the production of equivalent alternative and cheaper medicines (Attaran *et al*., 2012). The WHO in January 2016 updated and modified their definition to include substandard, spurious, falsely labelled, falsified and counterfeit (SSFFC) medical products (WHO, 2016). The WHO now defined a SSFFC medical product, as one that may contain no active ingredients, the wrong active ingredient, or the wrong amount of the correct active ingredient. In general, SSFFC medicines can be distinguished into the following five subtypes (Höllein *et al*., 2016):

i) Copies of the genuine brand medicines usually with correct amounts of the Active Pharmaceutical Ingredient (API).
ii) Products with the wrong API's; these can be poor or acceptable quality.
iii) Preparations containing no API at all.
iv) Medicines with too high or too low contents of the declared API.
v) Contamination of products with known and/or unknown purities.

However, it may not always be possible to discretely assign a sample to one of these subtypes (Hölllein *et al*., 2016). For example, products may contain trace amounts of API alongside an incorrect API and may also be contaminated with other material.

The use of the term SSFFC has now been superseded and from May 2017, the WHO amended their definition to Substandard and Falsified (SF) medical products. It is thought that the change simplifies the situation, as it was felt that the term SSFFC often confused the phenomenon with IP rights (WHO, 2017a). The term substandard is defined by the WHO as "out of specification authorised medical products that fail to meet either their quality standards, specifications or both" (WHO 2017a). The individual country's national regulatory medicine authority determines the quality standards and specifications that a product must meet. A medicine is deemed falsified if the "authorised manufacturer deliberately fails to meet these quality standards or specifications due to misrepresentation of identity, composition, or source" (WHO, 2017a). The change in definition by the WHO is seen as an "important and crucial milestone" in the prevention and detection of these medical products, it is thought that the definition adds clarity and simplification to an already complex issue (WHO 2017a). The WHO hope that member states and stakeholders adopt the change in definition to permit a more accurate comparison and analysis of data to combat this problem.

This change occurred in the latter stages of this research and the terms 'substandard and falsified (SF)' will now be adopted. However, several studies and articles were published before this

change and use the term counterfeit. Similarly, most referenced material includes the word 'counterfeit' and there is no clear distinction between substandard or falsified materials.

1.3 The Extent of the Problem

The falsification of medicines is a global phenomenon that affects lower, lower middle and high-income countries. In 2015, Mackey *et al.*, investigated incidents of counterfeit medicines penetrating the global legitimate supply chain. The study analysed data from the Pharmaceutical Security Institute (PSI) Counterfeit Incident System (CIS) over a 36-month period, 2009-2011. It discovered that globally there were 1,510 reports of counterfeit incidents, of which 1,799 different counterfeit medicine detections entered the legitimate supply chain (Mackey *et al*., 2015). The report found that China was responsible for approximately a quarter of detections (27.6%) in the legitimate supply chain. The top five countries represented a total of 65.7% of detections, which consisted of China (27.6%), Peru (11.6%), Uzbekistan (10.9%), Russia (8.4%) and Ukraine (7.2%) (Mackey *et al*., 2015). Other studies have been carried out; the results of which tend to be limited to individual therapeutic classes for example cardiovascular and antiparasitic (Kelesidilis and Falagas, 2015).

There is considerable literature to be sourced from investigative journalism (Bogdanich and Hooker, 2008; Bosely, 2011; BBC, 2011; Baibhav, 2016; Pitts, 2016 and Piervincenzi, 2016), with little public health enquiry relative to the size of this criminal enterprise (Cockburn *et al*., 2005). Details of these enquiries can be excessively delayed or supressed for legal, political, communication and economic reasons (Mackey *et al*., 2015).

Several systematic reviews of the literature have been reported, with some analysing a single therapeutic class (Almunzaini *et al*., 2013) and others spanning a broad spectrum of classes (Alghannam *et al.*, 2014). The study by Alghannam *et al*., (2014), analysed studies that used chemical identification/ quantification of an API. The group found that few studies reported evidence of counterfeits medicines (8%) whilst 55% of studies reported instances of substandard medicines, with the most reported problem adherence to specified API limits (Alghannam *et al*., 2014). A study by Koczwara and Dressman (2017), debated the figure reported by the WHO in 2006 for the prevalence of counterfeits globally, but concluded based on scientific literature from the last decade, that it was impossible to make reliable estimations on the prevalence of counterfeit medicines globally due to a lack of data. The group also found that the terms counterfeit and substandard were used synonymously and that the terms were not sufficiently differentiated between in the literature, which added further complexity to the issue (Koczwara and Dressman,

2017). This confusion between the terms counterfeit and substandard has also been reported by Grech *et al*., (2018).

The WHO estimates that a 30 – 40% of all medicines in lower and lower-middle income countries are substandard and falsified (Lui and Lundin, 2016) and up to 1% of medicines available in high income countries are likely to be, with this figure rising to 10% globally (Rebiere *et al*, 2017). It has also been reported that in some former Soviet Union countries, the figure for counterfeit medicines is as high as 20% of the pharmaceutical market (IMPACT, 2008). Data from the PSI has shown that, globally over the period of 2005 to 2010, the theft of pharmaceuticals increased 66% whilst counterfeiting instances increased 122% (Mackey *et al*., 2015). A current assessment in Kenya (Tanna *et al*., 2017) suggested that the level of substandard and falsified medicines was between 40 – 50% of the total with substandard material being the greater component (Tanna *et al*., 2017).

The effects of counterfeit medicines on patients range from the difficult to detect and quantify, to an important cause of unnecessary morbidity, mortality and loss of public confidence in medicines and health systems (Cockburn *et al*., 2005). Substandard and falsified medicines are usually first identified when problems with the medication are identified by the public, often resulting in an adverse reaction or a fatality. A study by Newton *et al.,* found that counterfeiters are able to produce high quality replica holograms (Newton *et al*., 2008), by purchasing them from specialist suppliers at competitive prices, the internet also enables the holograms to be obtained rapidly.

High-income countries are not immune from this problem; Pfizer have reported that 80% of Viagra purchased from the internet was falsified (Rushe, 2013) and in the United States of America (USA), there is a growing problem of fake medicines. In the UK healthcare is provided by the National Health Service (NHS) and is highly regulated, with all medicines monitored and licensed by the Medicines and Healthcare Products Regulatory Agency (MHRA) and European Medicines Agency (EMA) (NHS, 2014). Medicines are procured for the NHS through a national buying group (UK Government, 2017), meaning that the supply chain is tightly controlled. Medicines are available to patients via the NHS in three different ways, these are:

- Prescription Only Medicines – patients need a prescription issued by a Doctor or suitably qualified healthcare professional, these are available via a pharmacy at a fixed price or no cost to the patient,
- Pharmacy Medicines – available from a pharmacy, but under the supervision of a pharmacist,
- Over the Counter Medicines – can be purchased without the supervision of a pharmacist and available on general sale.

However, despite this, substandard and falsified medicines still occur in both the NHS supply chain and those sold on the internet. In many countries, none of these controls exist and medicines can be acquired from pharmacies, stores or "tuck shops" with no reference to a clinician or prescription.

1.3.1 Lower and Lower Middle-Income Countries (LIC and LMIC)

The counterfeiting of medicines is a major issue and has a profound impact on lower income countries (LIC) and lower middle-income countries (LMIC) (Almuzanini *et al*., 2013); these countries also bear the brunt of the problem (Bandiera and Marmo 2017). In this area of the world counterfeiters target lifesaving medicines, as these offer a higher profit (WHO, 2010b). This is because the demand for these medicines outweighs the financial ability of the country to pay. The most commonly targeted medicines in developing countries are antimalarials, antituberculosis, antiretrovirals and antibiotics (Höllein *et al*., 2016).

A United Nations Office on Drugs and Crimes (UNODC) report in 2009 stated that there were 83 million cases of treatment with antimalarials in the private sector, if half of these cases were treated with counterfeit medicines it would represent a market worth just under half a billion US dollars (UNODC, 2009). The same study also found that the ramifications of trafficking inert or substandard medicines into Western Africa were vast and extended beyond the region. However, due to lack of legislation and regulations, the counterfeiting of medicines is not generally regarded as a major organised crime activity (UNODC, 2009).

According to a report by INTERPOL, the deaths of six million people a year can be attributed to malaria, tuberculosis and HIV/AIDS (Selgelid, 2007). The International Policy Network (IPN) estimates that counterfeit antimalarial and antituberculosis drugs cause 700,000 deaths a year (Harris, 2009). Counterfeit antimalarials are of particular concern amongst healthcare bodies; the Food and Drug Administration (FDA) suggests that more than half the antimalarials in Africa are counterfeit (Atemnkeng *et al*., 2007). The New York Times reported that due to the scale and severity of the disease, malaria kills more than 2,000 children a day in Africa (Fuller, 2009). A report by INTERPOL also estimated that 200,000 children die annually after taking counterfeit antimalarial medication (Fenoff and Wilson, 2009).

Studies conducted by the WHO, UNODC and the Wellcome Trust found several instances of counterfeit antimalarials reaching the legitimate supply chain. These include, but not limited to

- In 1999, at least 30 people died in Cambodia after taking counterfeit antimalarials

prepared with sulphadoxine-pyrimethamine (SP) (which is an older less effective anti-malarial, that is still in use in some countries for example Zimbabwe, 2016), which was sold as artesunate (a water-soluble derivative of artemisinin) (Mukhopadhyay, 2007).

- In 2001 in South East Asia, out of 104 antimalarial drugs that were on sale in pharmacies, 38% did not contain any active ingredients (Mukhopadhyay, 2007).

A joint operation (Operation Jupiter) in 2008 between the WHO, INTERPOL, the Wellcome Trust and Chinese authorities, found that half of all artesunate tablets were counterfeit in South East Asia (Brierley, 2008). Of those found to be counterfeit, most tablets contained no artesunate, of which some contained a potentially toxic range of chemicals replacing the active ingredient (Brierley, 2008). Another case that demonstrates the danger of counterfeit medicines containing lethal ingredients is the death of 500 children in Bangladesh after ingesting paracetamol containing a renal toxin (diethylene glycol) (Newton *et al*., 2010).
Surveys conducted in South East Asia found that between 33% and 53% of artesunate purchased from pharmacies were counterfeit, containing either no or sub therapeutic quantities of the active pharmaceutical ingredient (API) (Newton *et al*., 2008). Studies also found that some counterfeit antimalarials contained active ingredients for other conditions; one such example is Sildenafil, the active ingredient in Viagra (Newton *et al*., 2001). Ricci *et al*., (2008) analysed the antimalarial artesunate by Raman spectroscopy and mass spectrometry and found that samples contained other APIs, these included acetaminophen, artemisinin, dipyrone, erythromycin and erucamide (Ricci *et al*., 2008).

Counterfeit medicines aggravate the growing problem of drug resistance (Atemnkeng *et al*., 2007 and Newton *et al*., 2008); the use of sub therapeutic levels of API is fueling this issue. Whilst the low amounts of API are high enough to pass qualitative screening tests, the dosages are too low to be effective. This exposure to sub therapeutic doses results in the malaria parasite becoming resistance to particular classes of drug (Brierley, 2008; BBC, 2012b and Noedl *et al*., 2008). Low API concentrations kill the weaker strains of the parasite but allow survival of more resistant mutations; thereby accelerating the evolution of the resistant strains.

There are several reasons for the growth of counterfeit medicines in LIC and LMIC. It is thought that inadequate access to health services, health insurance and the availability of a reliable pharmaceutical supply contribute to the problem (Mukhopadhyay, 2007). There is also the healthcare dilemma of quality (few tablets at a prohibitive cost) versus quantity (high volume of tablets for a low cost/ quality), often with practitioners opting for quantity. This is matched by

Governments who need to be seen to be doing something to provide healthcare for the population. Another reason may be due to the lack of monitoring of medicines, due to the relatively immature healthcare systems, restricted laboratory capacity, weak analytical infrastructures and chaotic logistical distribution (Höllein *et al*., 2016 and Kovacs *et al*., 2014). Counterfeits are more prevalent in countries where regulatory oversight is weak and where there is a minimal fear of prosecution. A report by the UNODC quoted the head of corporate security at Novartis as "If you get caught with a pound of cocaine, you can expect to do serious time. But if you are found with counterfeit medicines, you might do only six months." (UNODC, 2009). This emphasises the point of minimal fear of enforcement and prosecution.

Counterfeiters take advantage of the high cost of medication (IMPACT, 2008); in LIC, the cost of medicines can consume a significant portion of an individual's income incentivising patients to seek a cheaper alternative. It is common for some pharmacies in Africa to stock a cheaper substandard drug copy next to the original or generic brand (Atemnkeng *et al*., 2007). In Nairobi in 2017, different atenolol and metformin hydrochloride tablets with significant differences in cost were purchased. Unlike in high-income countries (HIC), patients in LIC rarely have health insurance or access to subsided healthcare system, which encourages the stocking of products at reduced costs.

1.3.2 High Income Countries (HIC)

In contrast to LIC and LMIC, lifestyle drugs are more commonly targeted in HIC (Russo *et al*., 2016). The most common counterfeited drugs are weight loss, anti-erectile dysfunction and cholesterol lowering drugs (MHRA, 2011a). Although the occurrence of counterfeit medicines reaching the legitimate supply chain in the UK via the NHS is low (ca 1%), the consequences can still be potentially life threatening (Jackson *et al*., 2012). This situation is similar in the USA (PSI, 2016).

Substandard and falsified products can affect both medicines and medical devices; the most common dosage form that is counterfeited are tablets. This is because tablets are the most popular and common route of oral drug administration (Jaimini and Rawat, 2013).

There have been several recalls of medicines found to be counterfeit in the UK between 2004 and 2009, of which four products had reached both pharmacy and patient levels and the other products were intercepted at wholesale level (MHRA, 2011a). Table 1.1 highlights recalls and interceptions that have occurred in several HIC.

Table 1.1: Recalls and interceptions relating to counterfeit medicines (Almuzani, 2013; European Compliance Academy, 2016; MHRA, 2011a and US Attorney 2014, 2016a, 2016b)

Year	Country	Product	Treatment
2004	UK	Cialis	Erectile Dysfunction
2004	UK	Reductil	Weight Loss
2005-2006	UK	Lipitor	Cholesterol Lowering
2007	UK	Zyprexa	Schizophrenia and Bipolar Disorder
2007	UK	Plavix	Prevention of Blood Clots – Cardiac
2009	UK	Seretide	Asthma
2010	USA	Tamiflu	Antiviral
2011	UK	Nurofen Plus	Pain relieving
2011	UK	Truvada	Antiretroviral
2011	UK	Viread	Antiretroviral
2012	USA	Avastin	Anticancer
2013	UK	Unknown	Type 2 Diabetes
2014	Germany	Sutent	Anticancer
2014	USA	Unbranded	Antiretroviral
2015	USA	Botox	Cosmetic
2015	USA	Unbranded	Anticancer
2015	Germany	Tenofavir	Antiretroviral
2015	Germany	Humira	Solution for pre-filled syringes
2016	UK	Medikinet	ADHD
2016	Germany	Cialis	Erectile Dysfunction

During 2005 to 2006, particular batches of Lipitor, a cholesterol lowing medicine, manufactured by Pfizer, were found to contain counterfeit material; stocks of Lipitor were removed from sale. The counterfeit batches carried the same batch number as a genuine Pfizer batch (MHRA, 2011a). In May and June 2007, batches of Plavix, a cardiac medicine, manufactured by Sanofi-Aventis and Bristol Myers Squibb, were found to have been repackaged into English language cartons, which displayed the same batch numbers as those that were intended for the French market and packaged with French livery (MHRA, 2011a), Plavix was withdrawn from sale.

It is not just Prescription Only Medicine's (POM's) that are counterfeited; in August 2011, Nurofen Plus, a pain-relieving medicine that is available Over the Counter (OTC), was recalled due two or more batches containing the wrong API (BBC, 2011). Batches affected were found to contain Seroquel, an anti-psychotic medicine and Neurontin, an anti-epileptic medicine (BBC, 2011 and MHRA, 2011a).

In other countries, instances of substandard and falsified medicines are increasing and this trend looks to continue, whether this is the result of detection methods becoming more advanced and therefore able to detect more occurrences, or if the counterfeit market is growing. In America cases of counterfeit medicines almost doubled (30 cases to 58 cases) in 2003 and 2004 (Wertheimer and Norris, 2009). In the past decade in Australia, there have been 6,000 seizures of

counterfeit Viagra, the seized tablets contained, but not limited to contaminated paint, heavy metals, rat poison, chalk and anti-freeze (Bandiera and Marmo, 2017).

1.3.3 Internet Pharmacies

Due to the popularity of people 'self-diagnosing' using the internet, individuals are now buying medicines at the same time to complement their diagnosis regardless if it is correct, reinforcing the illusion of the internet being cost effective and time saving. Because of this, it is becoming more and more common for counterfeiters to use the internet as a means for selling and distributing counterfeit medicine. In the last decade in the UK, there has been rapid expansion in the number of websites offering medicines for sale via the internet (MHRA, 2011a). Surveys conducted by the WHO and other regulatory bodies have found that around 50% of medicines purchased on the internet from websites that conceal their physical address are counterfeit (EAASM, 2012).

There has been an increase in the UK in recent years of people buying prescription drugs online (BBC, 2010). These flourishing internet pharmacies have transformed the conventional method of medicine distribution (Lui and Lundin, 2016); allowing people in the UK to buy anything from lifestyle to lifesaving medicines, often without prescription. These unregulated websites, further facilitate the availability of counterfeit medicines (Lui and Lundin, 2016). The risk in the UK is that many of these websites originate from abroad and are therefore not subjected to British regulations (MHRA, 2011a). In 2015, the police and MHRA raided the premises of a UK online pharmacy, 60,000 units of potentially dangerous medicines worth an estimated 2.4 million US dollars were seized (INTERPOL, 2015). In 2010, it was estimated that the European counterfeit drugs market was worth 10.5 billion Euros each year, with 1 in 5 Europeans buying counterfeit medicines (Grogan, 2010). It was also believed that as many 2.5 million men in Europe had taken counterfeit Viagra (Gardner, 2010). This has resulted in an annual increase in sales of counterfeit medicines of 15%; evidence of this includes the seizure of 11.4 million counterfeit medicines at EU borders in 2009, representing a 422% increase since 2006 (Mackey and Liang, 2011).

The European Alliance for Access to Safe Medicines (EAASM) investigated the purchase of POM from the internet (Fake Britain, 2010). Results showed that not only could POM's be easily purchased, but also an alarming lack of patient care or information was provided. Examples included supplying free Viagra with cardiac medication, counterfeit Lipitor and supplying a hair loss drug that can cause infertility in women to a female recipient without any checks taking place (Fake Britain, 2010). In the UK, all the medicines that were obtained by the programme required a medical check by a doctor and a prescription to purchase them at a pharmacy or a registered

online pharmacy. However non-registered online pharmacies do not require a valid prescription or any form of medical history to supply a POM. It is illegal in the UK to obtain a POM without a valid prescription (UK Government, 1997) and could have life threatening consequences for the patients.

The International Medical Products Anti-Counterfeiting Taskforce (IMPACT), a joint venture between the WHO, INTERPOL and other stakeholders established in 2006; believe that companies whose websites conceal their real identity sell counterfeit medicines (IMPACT, 2008). These companies tend to be international, sell POM's without a valid prescription and often deliver products with unknown origins (IMPACT, 2008). In June 2015, INTERPOL carried out Operation Pangea VIII, an annual operation targeting the online sales of medicines and medical devices (INTERPOL, 2015). A record 20.17 million units of medicines were seized in just one week, worth 81 million US dollars (Lundin, 2015). These medicines included blood pressure, anti-erectile dysfunction and cancer medication, as well as nutritional supplements (INTERPOL, 2015). In the UK, 6.2 million units of medicines were seized worth £15.8 million (Gallagher, 2015).

Nevertheless, non-registered internet pharmacies are on the increase (Fittler *et al*., 2013); the main driver is the growth of the internet, which stimulates the growth in internet pharmacies. In particular, POMs can be purchased from non-registered internet sites without prescriptions, creating a fake belief that time and money can be saved without having to visit a doctor and often to avoid embarrassment for some complaints.

1.3.4 Generic Medicines

The usage of generic medicines can complicate the already complex issue of substandard and falsified medicines. Generic medicines enter the market after the patent expires on the innovator product. In order for a generic formulation to obtain approval from the relevant regulatory body, the formulation must be validated and shown to be bioequivalent and biopharmaceutically equivalent to the innovator product (Dharmalingam *et al*., 2014). It must also be identical in API release profile, purity, quality, dosage form and route of administration (Olusola *et al*., 2012).

The introduction of generic medicines into health care systems, in particular in LIC and LMIC is aimed at reducing costs and therefore improving the overall delivery of healthcare in these countries (Olusola *et al*., 2012). However, the quality control (QC) and effective monitoring of generic medicines in LIC and LMIC is often absent. The QC issue itself raises concerns with the distribution of substandard and falsified medicines (Dharmalingam *et al*., 2014), with some

sources quoting that substandard medicines pose a greater threat to public health than falsified medicines (Odeniyi *et al*., 2003 and Davidson, 2011).

Therefore, it is important that the relevant healthcare agencies and regulatory bodies monitor the quality of generic formulations. It is thought that effective monitoring of medicines can lessen the financial burden on countries caused by health issues, resulting from the usage of substandard and falsified medicines (Dharmalingam *et al*., 2014 and Blackstone *et al*., 2014).

Studies in Kuala Lumpur and Nigeria assessed the quality of generics against the innovator brand for atenolol and metformin hydrochloride tablets using the methodologies from the British Pharmacopeia (Dharmalingam *et al*., 2014 and Olusola *et al*., 2012). The aim of the studies was to evaluate and establish the quality of most used generics against the innovator product (Dharmalingam *et al*., 2014 and Olusola *et al*., 2012). Both studies used a "working reference standard", which was an analytical grade API powder, as opposed to a reference tablet. The studies established that all of the atenolol tablets analysed passed the pharmacopeia tests and were found to be bioequivalent to the innovator drug; whereas only four of the eight metformin hydrochloride brands tested could be classed as bioequivalent and passed the relevant pharmacopoeia tests (Dharmalingam *et al*., 2014 and Olusola *et al*., 2012). These studies showed that there is variation amongst generic formulations and some could even be classed as substandard, as not all of the metformin hydrochloride generics met the specifications outlined by the pharmacopeia.

1.4 Growth of the Substandard and Falsified Medicines market

Several factors make falsifying medicines attractive. Counterfeiting is a lucrative business, medicines are high value items in relation to their bulk and the demand for medicines is continually increasing (WHO, 2011). There are greater profits to be made in falsifying medicines than from smuggling Class A drugs (WHO, 1999); also, the penalty if caught is considerable less than that of Class A substances. The cost of ingredients can be very low if expensive active ingredients are omitted or cheap substitutions used (WHO, 2011; Kelesidils and Falagas, 2015).

Legitimate pharmaceutical manufacturers must ensure product quality and are carefully regulated, which results in considerable overheads. Counterfeiters have little quality assurance and no regulation to adhere to; hence their profit margins are very high (WHO, 2011; Krakowska *et al*., 2016).

The production of falsified medicines does not require large infrastructure or facilities; many operate as cottage industries (IMPACT, 2008), often using 'ingredients to hand'. Contaminants

and adulterants vary from batch to batch. Jackson *et al*., (2010) reported contaminants used in the falsification of Viagra tablets seized in the UK; these included caffeine and bulk lactose in one batch and in another subset talcum powder, commercial grade paints and API's for other pharmaceutical products (paracetamol and metronidazole) were identified. The group also reported that in some cases printer ink was used to produce the blue colour of the tablets (Jackson *et al*., 2010). Furthermore, counterfeiters have also been found to use pharmaceutical companies that manufacture genuine medicines during business hours and at night manufacture counterfeits (Mukhopadhyay, 2007). Instances in China, where a large proportion of counterfeits are manufactured, have shown that counterfeiters take advantage of European and US patent laws not being recognized to manufacture medicines which are classed as legal in China. These medicines only become illegal once they are exported (MHRA, 2011b).

Counterfeiters have also set up companies providing a service to dispose of expired medicines, only to repackage and resell them (Mukhopadhyay, 2007; Kamba *et al*., 2017).

The falsification of medicines is greater in those regions were the regulatory oversight is weak. In some countries, the counterfeiting of medicines is not treated as a criminal offence, as there is no legislation or regulations that exist for the proper control of medicines (WHO, 2011). Where sanctions are imposed on counterfeiters these are often not a significant deterrent and create a minimal fear of prosecution (WHO, 2011).

The growth in the international trade of pharmaceutical ingredients and medicines only adds another complexity to this issue (Consoli, 2010). As many pharmaceutical companies seek to outsource production of excipients and/ or medicines to reduce costs, the quality and purity of these can be compromised and the risk of counterfeiters accessing the supply chain increases.

1.5 Combating Substandard and Falsified Medicines

Healthcare and regulatory bodies are now working with governments to implement new laws and task forces to combat the problem of counterfeit medicines.

In America, the Counterfeit Drug Enforcement Act 2014 was introduced in December 2014 however it was not enacted, with previous revisions of the act also being rejected by congress; this act seeks to increase penalties for the sale and trade of counterfeit drugs (Anon, 2014). The FDA also have a voluntary agreement with pharmaceutical companies, in which companies are expected to report suspected counterfeit medicines to the FDA within 5 days of discovery (Food and Drug Administration, 2009).

The European Union (EU) has issued directives of the European Parliament to combat the issue of counterfeit medicines. In 2011, the directive 2001/83/EC relating to medical products for human use and prevention of entry of falsified medicinal products into the supply chain was amended and now requires all member states to have a system in place to detect such medicinal products. This involves introducing an electronic system containing registration details of manufacturers, importers and distributors (Merks *et al*., 2016). Further to this in 2016, a new European Commission (EC) regulation (EC 2016/161) dictates that by February 2019, all member states must have implemented two safety features on human medicine packaging. These include a unique identifier and an anti-tampering device (European Medicines Agency, 2016). The directive mandates that all POM's be serialised with a unique code printed on the packaging that can be verified and authenticated at the point of supply to the patient (Naughton *et al*., 2016)

In 2009 in Kenya, the Anti-Counterfeit Act 2008 became law clamping down on fake products. Critics believed that this act would blur the lines between generic, substandard and counterfeit medicines. One such critic is Médecins Sans Frontières (MSF), who believes this anti counterfeit measure could potentially deprive Africa of cheap medicines (Chatterjee, 2010). MSF depend mainly on low cost generic versions of essential medicines to treat patients around the world. In 2010, the Constitutional Court in Kenya barred its Government from implementing the new law, as the law applied to generic medicines (Gathigah, 2010). For many years, the newer antimalarial medicines were not used in Kenya, because the healthcare authorities could afford only generic medicines and these might have been considered counterfeit. This unusual situation has been rectified and discussed at length by the Kenyan Government. Petitioners to the new law believe the act confuses quality and intellectual property rights, therefore making legitimate generic drugs counterfeit (Gathigah, 2010). This is due to the term counterfeit being deemed controversial as discussed earlier in this chapter.

Other East African countries look to follow Kenya, Uganda has a draft anti counterfeit bill and Tanzania, Rwanda and Burundi are currently in talks over these issues.

There is still significant resistance to discuss the occurrence of counterfeit or falsified medicines. For example at the recent 15th International Association of Therapeutic Drug Monitoring & Clinical Toxicology Conference in Kyoto, Japan, there was not a single reference to counterfeit or falsified medicines. In fact, several delegates indicated that their national governments discouraged such terms.

2 Tablet Formulation

The most common oral dosage form is the tablet, the manufacturing process and formulation of the tablet can vary depending on the medicine. Excipients are the main constituents of a tablet, excluding the API and are often included to aid manufacture, administration or adsorption and even enhance the colour of the tablet. Excipients can vary between the innovator product and generic formulations, however any excipient used in the product is tightly controlled by regulatory bodies.

Illnesses of global concern were identified (cardiovascular disease and diabetes) and appropriate tablet formulation medicines were acquired opportunistically from around the world. The overall aim for this research was to examine a range of analytical methods, which could identify and potentially quantify the API present in a single tablet. Chapter 3 discusses a range of analytical techniques and assesses the ability to detect SF medicines.

2.1 Selection of Target Medication Forms

All healthcare providers look to supplying optimum care in the most cost-effective way.

The oral route of drug administration is the most common and frequently used with tablets accounting for the majority of all dosage forms (Jivraj *et al.*, 2000; Yasmeen *et al.*, 2005; Jaimini and Rawat, 2013). The cost, convenience of dosing and stability compared to other dosage forms, controlled release of the API, allowance of the incorporation of more than one API and the ability to self-administer, make tablets the most popular dosage form (Jivraj *et al.*, 2000; Jaimini and Rawat, 2013). This research has therefore focused on developing qualitative and quantitative methodology for the rapid screening of tablet formulations for the presence of substandard and falsified medicines.

2.2 Components of a Tablet

The British Pharmacopeia (BP) define tablets as ‘solid preparations each containing a single dose of one or more active substances. They are obtained by compressing uniform volumes of particles or by another suitable manufacturing technique… The particles consist of one or more active substances with or without excipients.’ (British Pharmacopeia, 2017a).

Excipients are the main constituents of a dosage form excluding the API (European Commission, 2003). They are often included to aid manufacture, administration or adsorption (Crowley and

Martini, 2001). Excipients are sometimes included for other reasons, such as product differentiation, appearance enhancement or retention of quality (Crowley and Martini, 2001).

The selection of excipients within a tablet formulation plays an important role in the overall quality of the product (Haywood and Glass, 2011); along with the grade and quality of the excipients and the drug manufacturing process (Srinivasan, 2014). This is significant, as many pharmaceutical companies outsource the manufacture of their products to reduce overheads and production costs. This can sometimes result in a poorer quality product, often substandard, as some countries do not have robust quality control measures compared to the UK, Europe and America.

The range of excipients used in the top 200 prescription tablets, in the USA, was investigated (Dave 2008), and the results are shown in Figure 2.1. For simplicity, chemically similar and hence spectroscopically similar, excipients have been grouped together. These are:

- Cellulose: MCC, methyl, ethyl, HPMC, cellulose and carboxymethylcellulose.
- Calcium Phosphate: dibasic, tribasic, anhydrous, diphosphate and hydrogen phosphate dehydrate.
- Cross povidone: povidone and PVP.
- Lactose: hydrous, anhydrous, monohydrate and spray dried.
- Silicon dioxide: colloidal
- Starch: corn, potato, sodium, and pregelantised.
- Carbonate: magnesium and calcium.

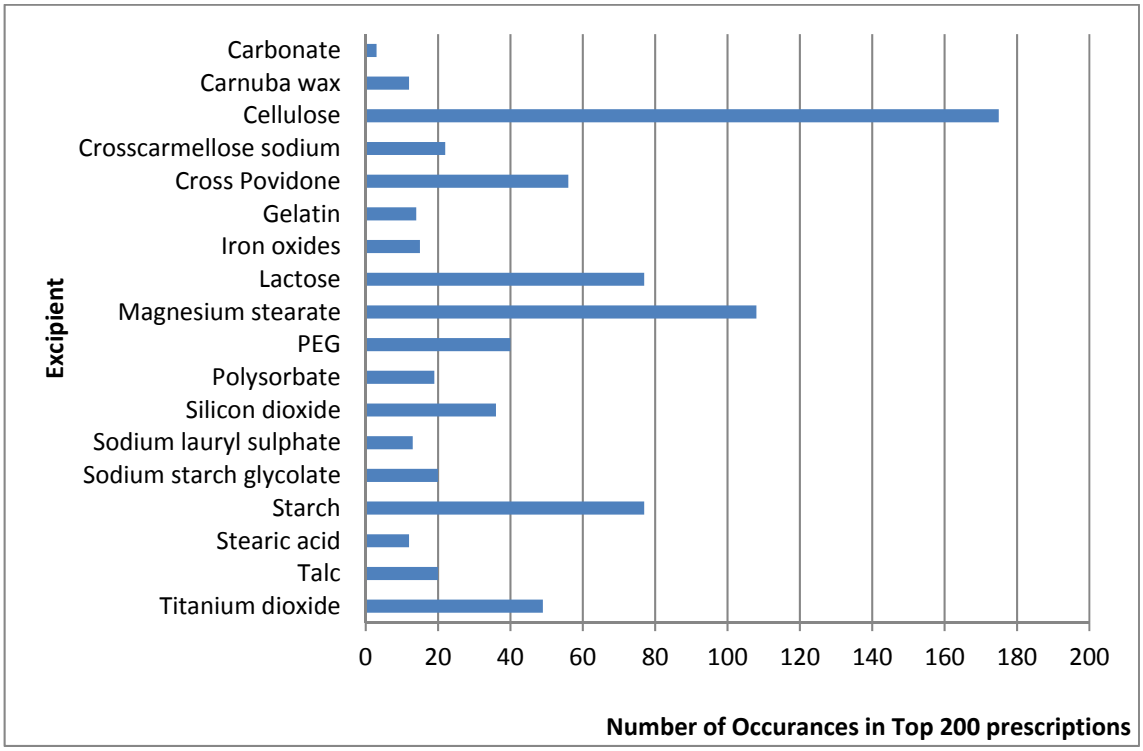

Figure 2.1: Frequency of excipient usage for the top 200 tablets (Dave, 2008)

The excipients shown in Figure 2.1 can be categorised into the following types as summarised in Table 2.1.

Table 2.1: Different excipient components of typical tablet formulations

Excipient	**Amount in Tablet (%)**	**Mg/Tablet**
Diluents (Fillers)	5 – 80	20 – 400
Binding Agents	0.5 – 5	2 – 20
Lubricants	0.3 – 2	1.5 – 10
Disintegrants	5 – 20	20 – 100
Glidants	< 0.2	< 1
Colourants (Pigments)	0. 1 – 0.5	< 2
API	2 – 85	1 – 500

It is important to note that generic formulations of the innovator product may differ in the excipient usage. Whilst the physical appearance and bioavailability is tightly controlled (Food and Drug Administration, 2015), manufacturers may use different excipients from those in the innovator product; however, any excipient used in the proposed formulation must be included in the FDA Inactive Ingredient Database (IID or IIG) in the USA (Srinivasan, 2014). In the UK, any

excipients used in the formulation must be approved by the MHRA or European Medicines Agency (EMA).

2.2.1 Diluents

Diluents are commonly used as fillers in a tablet and more often added as a bulking agent to aid compression and improve bulk powder flow (Mills, 2007). Diluents have a strong influence over powder flow and can enable blended powders to flow quickly (Mills, 2007). They are often carbohydrate based, but can also contain phosphates; common diluents include lactose, sucrose, starch, cellulose, microcrystalline cellulose (MCC) and dicalcium phosphate dihydrate (Aulton, 2007, Gohel, 2016a). The concentration of diluents in a tablet can range from 5% to 80% (Gohel, 2016a).

Lactose is the most common filler used in the manufacture of tablets (Aulton, 2007), with varying forms used in the different manufacturing processes. These include spray-dried lactose, which has been specifically designed for direct compression (Jivraj *et al*., 2000) and anhydrous lactose, which is commonly used in wet and dry granulation.

MCC is widely used in the pharmaceutical industry as a diluent, this because it acts as both a filler and a binder (Jivraj *et al*., 2000). Alongside spray dried lactose, MCC is one of the most useful fillers for direct compression (Jivraj *et al*., 2000), this is due to it exhibiting excellent compactability at low pressures, high dilution potential and superior disintegration properties (Jivraj *et al*., 2000).

2.2.2 Binding Agents

Binding agents are added to the tablet to act as an adhesive and bind together the powder and granules (Mills, 2007). Binders can be added either ‘dry’ or dissolved in granulation fluid (either solvent or water based) (Aulton, 2007). If a granulating fluid is used, it needs to be evaporated off, leaving the binder in place to adhere to the granules. Dry binders are often used in the direct compression process, as solutions cannot be used (Aulton, 2007).

The amount of binder added is important, as it affects the overall mechanical strength of the tablet (Aulton, 2007). Too much binder results in very hard tablets and an increase in disintegration time, with too little binder resulting in a weak tablet that may not withstand further processes.

Common binding agents are typically polymeric based, for example polyvinylpyyrolidone (PVP) and hydroxypropyl methylcellulose (HPMC) (Aulton, 2007). Others can include MCC, sucrose

and gelatin (Mills, 2007 and Aulton, 2007). The concentration of which binders are added is dependent upon the molecular weight, with concentrations ranging between 0.5% to 5% w/w.

2.2.3 Lubricants

Lubricants are used in the formulation of tablets, as they prevent the adherence of granules/powders to the punch die/faces and promote smooth ejection from the die after completion (Mills, 2007), therefore reducing the effect of pitting on the tablet surface. Most lubricants are hydrocarbon based and may be either insoluble or soluble in water.

Common insoluble lubricants include magnesium stearate and stearic acid, with magnesium stearate being the most extensively used tabletting lubricant (Mills, 2007). Soluble lubricants include polyethylene glycol (PEG) and lauryl sulphate salts (sodium or magnesium) (Aulton, 2007). The efficacy of PEG is less then magnesium stearate, so insoluble (hydrophobic) lubricants are often preferred.

The hydrophobic concentration level (typically 0.3% to 2%) needs to be optimised (Mills, 2007), as it can affect the tablet quality. If under lubricated, the blend flows poorly and can have compression sticking problems, whereas over lubricating can adversely affect the tablet hardness and dissolution rate (Mills, 2007). Effective lubrication alongside powder flow is important in the pharmaceutical industry for high throughput of product (Mills, 2007).

2.2.4 Disintegrants

Disintegrants are added to aid de-aggregation of compacted tablets (Mills, 2007). The presence of a disintegrant causes a more rapid break-up of the tablet upon exposure to moisture (Mills, 2007). It is important that the tablet be broken down, to facilitate dissolution in the gastrointestinal tract prior to absorption into the blood stream.

Disintegrants react on contact with moisture and in most instances water uptake alone will cause disintegration. This occurs when the interparticle cohesive forces that hold the tablet together are ruptured (Mills, 2007). Some disintegrants work by swelling on contact with moisture, in this case the tablets are broken down by physical rupturing, as the channels of penetration are widened, thereby increasing the rate of water uptake into the tablet (Mills, 2007).

The most common disintegrants are maize starch, sodium starch glycolate (Primogel) and celluloses, for example methylcellulose (Aulton, 2007). The concentration of these ranges from 5% to 20% (Gohel, 2016b).

2.2.5 Glidants

Glidants can enhance powder flow, but are not a standard excipient and are only added if necessary (Mills, 2007). Glidants can be difficult to work with, as they are very voluminous, therefore controlling the level of glidant can be challenging (Mills, 2007).

Glidants improve powder flow by adhering to particles, thus reducing interparticle function (Mills, 2007). They are most commonly used in dry powder formulations and in the direct compression manufacturing process.

The most common glidant used is colloidal silicon dioxide and is required in very low levels, usually less than 0.2% (Mills, 2007).

2.2.6 Colourants (Pigments)

Colourants are often used to achieve a certain visual effect and to a less extent to influence the mechanical properties of the coating (Aulton, 2007). Acceptable colourants for use pharmaceutically are available as both pigments (insoluble in water) and dyes (water-soluble). Pigments are often used in film-coated formulations, as they are more chemically stable; provide better coverage and a means of optimising permeability in film-coated formulations (Aulton, 2007).

The most common pigments used are iron oxides, titanium dioxide and aluminium lakes (Aulton, 2007). Lake colorants are formed by chemically reacting straight dyes with precipitants and salts. Lakes are often used as colour additives for tablet coatings due to their stability (FDA, 2003).

2.3 Tablet Formulations for this research

In Low and Middle Income countries, there has been a shift from infectious to non-communicable disease, as the leading cause of morbidity and mortality (Antignac *et al.*, 2017), with cardiovascular (CV) disease and diabetes a global burden (Al-Hamid *et al.*, 2016). In recent years there has been a transfer from HIC to LIC for CV deaths, in HIC the rate of deaths caused by CV disease has significantly decreased, whilst in LIC the frequency of CV deaths has been on the rise (Antignac *et al.*, 2017). It is thought that more than 80% of deaths globally from CV disease occur in LIC and LMIC (Antignac *et al.*, 2017). The WHO state that by 2020, the major sources of morbidity globally will be CV disease and diabetes (Al-Hamid *et al.*, 2016). Therefore, atenolol (cardiovascular disease), metformin hydrochloride (diabetes) and a range of antimalarials were selected for analysis.

The samples were acquired opportunistically via interested colleagues, PhD students and undergraduates studying on the Pharmacy and Pharmaceutical and Cosmetic Science courses at De Montfort University.

This research assesses the ability of various analytical techniques to detect/identify SF medicines in a single tablet. Standard assay tests by the British Pharmacopeia (BP) can often use ~20 tablets per assay (British Pharmacopeia 2017b); in reality this is not feasible in LIC and LMIC, as there are often not 20 tablets to spare per test, as the cost of medicines is expensive. When performing analysis of a batch, the concentration of API is often averaged out over the 20 tablets, the patient however only takes a single tablet, and any fluctuation in dosage would affect the patient. The purpose of this research is to assess the feasibility of identifying SF medicines from a single tablet analysis.

2.3.1 Atenolol

Atenolol [4-[2-hydroxy-3 isopropyl–aminopropoxy]-phenyl-acetamide] is a β1-receptor antagonist belonging to the β blocker category. Figure 2.2 shows the molecular structure of atenolol. Atenolol is primarily used for the treatment of cardiovascular diseases, including hypertension, arrhythmias and for longer-term management of angina pectoris (Khataee *et al*., 2016 and Dharmalingam *et al*., 2014)). The use of atenolol in sport is prohibited and is included on the World Anti-Doping Agency's (WADA) List of Prohibited Methods and Substances. This is due to atenolol being a misused as a doping agent, as it has a calming effect on the athlete (Farcaş *et al*., 2016 and Khataee *et al*., 2016). Although atenolol is used for the treatment of hypertension, it can lead to severe hypotension if taken excessively (Khataee *et al*., 2016).

Common excipients used in the formulation of atenolol tablets include, but not limited to calcium hydrogen phosphate dehydrate, silica colloidal anhydrous, magnesium carbonate, magnesium stearate, maize starch, MCC, crospovidone and titanium dioxide.

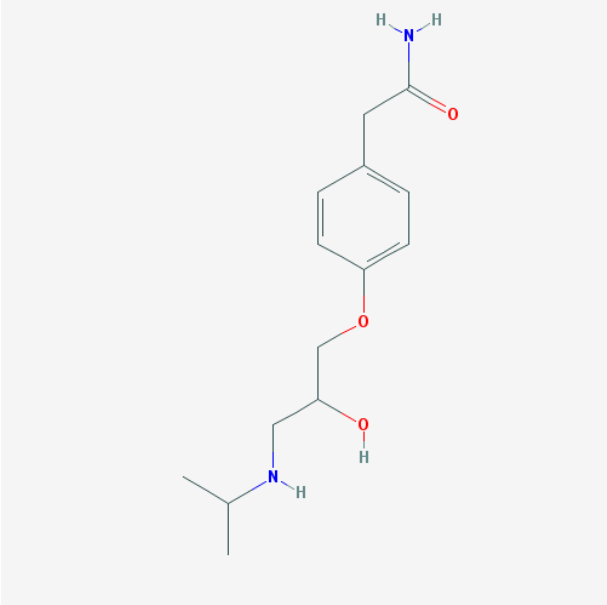

Figure 2.2: Molecular structure of atenolol

2.3.2 Metformin Hydrochloride

Metformin hydrochloride belongs to the biguanide class of drugs and is often used as the first line agent for the treatment of type 2 diabetes (Olusola *et al*., 2012). It is one of only two antidiabetic tablets included in the WHO's Model List of Essential Medicines, as it has very few adverse effects (Diabetes, 2017). It is often prescribed for the management of type 2 diabetes, particularly in cases where the patients are also overweight, obese or suffer from polycystic ovary syndrome (Olusola *et al*., 2012). Metformin hydrochloride works by supressing hepatic glucose production, increasing insulin sensitivity therefore enhancing peripheral glucose uptake. This then increases the oxidation of fatty acids and results in lower absorption of glucose in the gastrointestinal tract (Diabetes, 2017). Figure 2.3 shows the molecular structure of metformin hydrochloride.

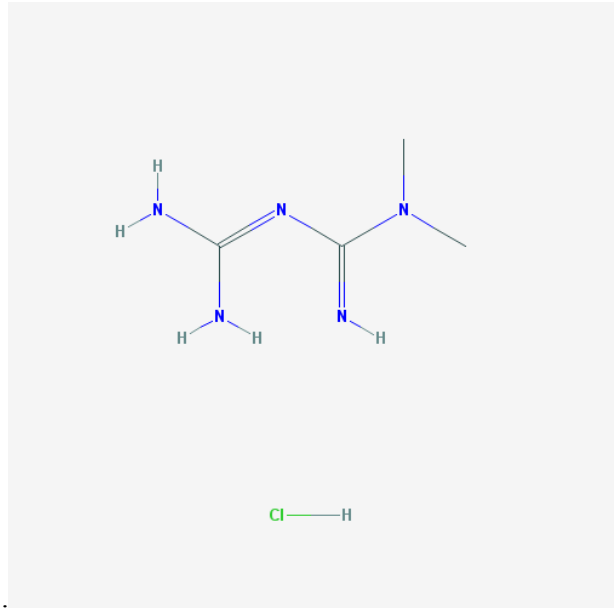

Figure 2.3: Molecular structure of metformin hydrochloride

Common excipients used in the formulation of metformin hydrochloride tablets include, but not limited to magnesium stearate, maize starch, povidone and sodium starch glycolate.

2.3.3 Antimalarials

Antimalarials are used in the prevention and treatment of malaria (NHS, 2015). Antimalarials are used to eliminate the *Plasmodium* parasite from the patient's blood. The WHO recommends artemisinin based combination therapies (ACT) for the treatment of malaria caused by the *P. falciparum* parasite, these include:

- Artemether and lumefantrine,
- Artesunate and amodioquine,
- Artesunate and mefloquine,
- Dihydroartemisinin and piperaquine,
- Artesunate and sulphadoxine – pyrimethamine (SP) (WHO, 2017b).

Chloroquine or ACT is used for infections caused by *P. vivax*, in chloroquine resistant areas quinine is used to treat the infection (WHO, 2017b). Figure 2.4 shows the molecular structure of chloroquine diphosphate and Figure 2.5 shows the molecular structure of quinine sulphate.

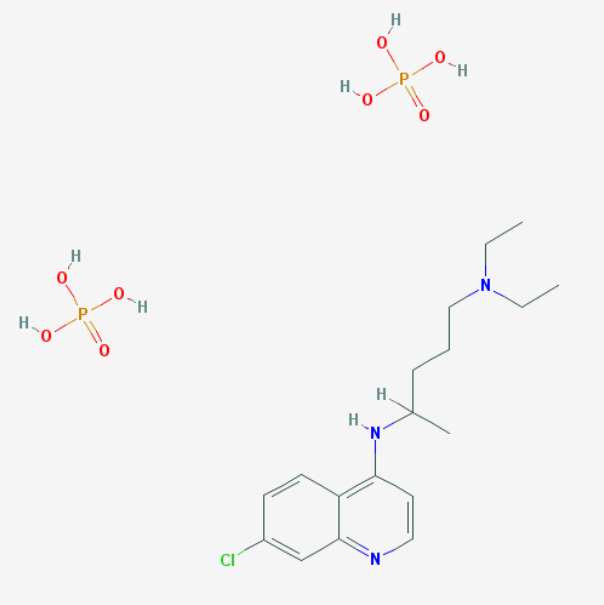

Figure 2.4: Molecular structure of chloroquine phosphate

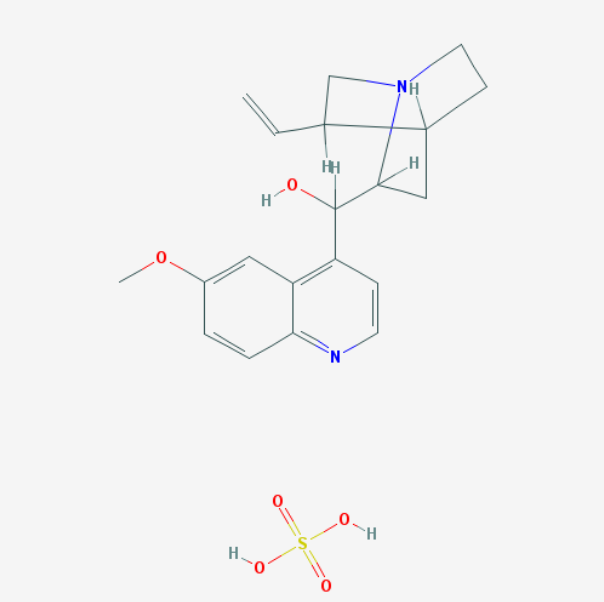

Figure 2.5: Molecular structure of quinine sulphate

Common excipients used in the formulation of antimalarial tablets include, but not limited to croscarmellose sodium, hypromellose, lactose monohydrate, magnesium stearate, maize starch, MCC, povidone, silicon dioxide and SLS.

2.4 Study Aims

All tablets have complex formulations, typically of three or more components and any suitable analytical method must be able to either

- Identify the analyte in a complex mixture

Or

- Selectively isolate the target analyte prior to identification.

In both cases, subsequent quantification would be advantageous.

The aim of this study is to examine a range of analytical methods, which can identify and possibly subsequently quantify the API's present in a single unknown tablet sample.

This can be achieved by the following objectives:

i) Systematic review of the literature.
ii) Assessment of analytical equipment for the ability to identify and quantify an API in a tablet, with respect to LIC's and LMIC's.
iii) To consider the reliability and validity of the equipment/ methods.
iv) Sample selection based on prevalence of disease.

3 Detection of Substandard and Falsified Medicines

A range of analytical techniques were assessed for their capability to detect SF medicines in particular the identification and quantification of the API.

Instrumental selection criteria for this study included the potential ability to use these techniques in LMIC's and the need for little or no sample preparation. Different analytical techniques require different levels of sample preparation as summarised in Figure 3.1. Some techniques were discounted on being beyond the scope of LMIC's and not fitting the criteria of single tablet capacity.

Spectroscopic, chromatographic and microscopic methods were assessed with relation to the criteria and ability to identify SF medicines. Chapter 4 outlines the methods and instruments selected to identify potential rapid screening methodologies.

Methods for detecting substandard and falsified medicines vary from 'low tech' approaches, for example visual appearance, Thin Layer Chromatography (TLC), Minilab and colourimetric tests, to those that are technically refined, for example Raman, Near Infrared (NIR) and Mass Spectrometry (MS). However, counterfeiters are fast to react to new methods to stop them (Mukhopadhyay, 2007), so the need to develop new robust techniques is important. A significant consideration for any new technique that is developed is that it can be employed in both HIC's and LIC's. Much of the infrastructure that is taken for granted in HIC's is absent or unreliable in LMIC and LIC's. In many parts of the world, power and water supplies can be uncertain and specialist analytical equipment and trained staff will be in short supply (Höllein *et al*., 2016).

Many counterfeiters will use a small amount of active ingredient in order to bypass some qualitative tests, for example TLC and colourmetric tests. Most techniques are often used in combination with others to build a profile, including chemical, biological and packaging evidence (Newton *et al*., 2008). This provides the correct identification at the sample screening stage, but provides insufficient dosage of the API to the patient, resulting in absence of any therapeutic benefit.

In this work, a rapid screening process capable of identification and quantification of an API will be investigated. Substandard and falsified medicines can be analysed by a variety of techniques; the techniques available fall into the following general categories and methodologies:

- Spectroscopic – light absorbance (IR, UV), light emission (Raman) at specific frequencies
- Chromatography – thin layer/ paper separation

- Mass Spectrometry – ionisation giving specific m/z values
- Microscopy – SEM and X-ray analysis for elemental composition
- Nuclear Magnetic Resonance – energy absorbance to give structural data

Whilst considering the range of techniques available the potential to use the technique in a LMIC with few trained staff and facilities must be kept in mind. Furthermore, techniques requiring little or no sample preparation would be preferred. Each of the techniques referred to above requires different degrees of sample preparation, ranging from non-destructive techniques to those needing crushing and or dissolution of the dosage form. Figure 3.1 summarises the level of sample preparation of medicines for some analytical techniques.

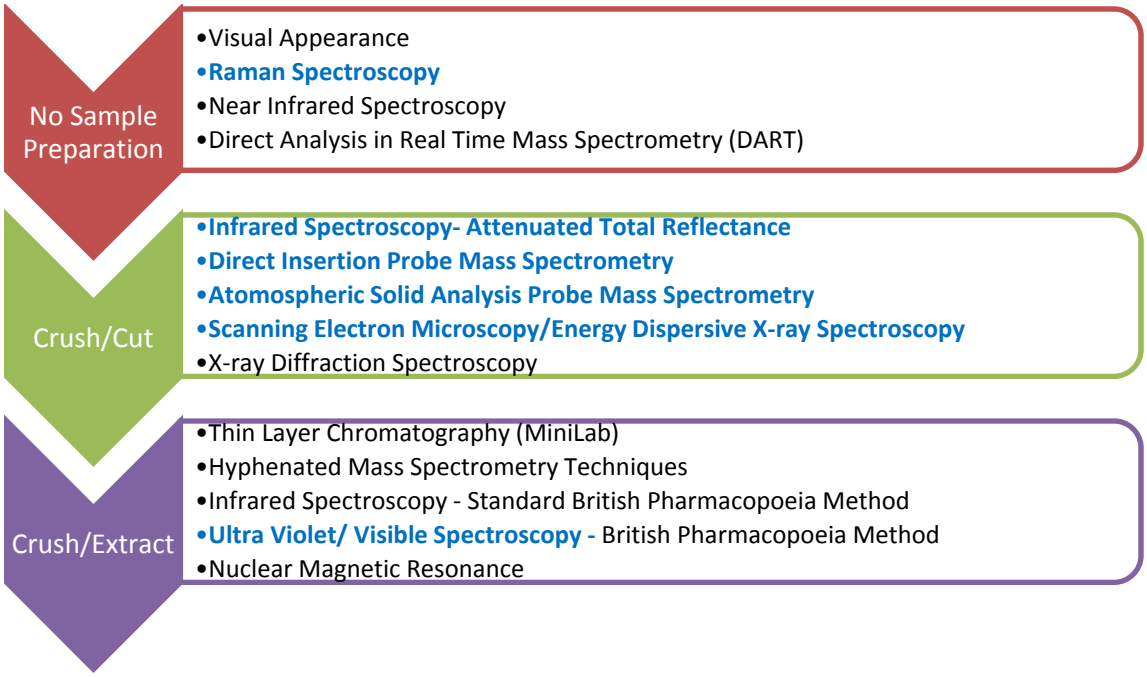

Figure 3.1: Levels of sample preparation for some analytical techniques, with those to be investigated highlighted

Sections 3.1 to 3.4 discuss both current and evolving techniques that can be used to analyse counterfeit medicines. The focus is on rapid data output generated by relative non-specialists. Within the criteria, information is needed that has a high degree of specificity to minimise the number of false positives identified with the analyst and the loss of revenue.

Table 3.1 summarises the advantages and disadvantages for the analytical techniques analysed.

Table 3.1: Advantages and Disadvantages for the analytical techniques analysed

Technique	Advantage(s)	Disadvantage(s)
UV	Quantification of the API.	Requires solvents to extract the API. Time Consuming.
ATR FTIR	Sample preparation is minimal. Can identify API's in a whole tablet formulation when compared to a reference. Able to quantify the amount of API.	Surface technique. Cannot analyse whole tablets. Mixing can be an issue.
Raman	Non-destructive, sample preparation is minimal if at all. Can analyse whole and powdered samples. Can identify API's in a whole tablet formulation when compared to a reference.	Some samples are prone to the effect of fluorescence, which can affect results. Requires multivariant analysis to quantify.
DIP MS	Sample preparation is minimal. Can identify the target API, as it is selective technique.	Difficult to quantify amount of API. Source can easily become contaminated, as inserted directly into it.
ASAP MS	Sample preparation is minimal. Can identify the target API, as it is selective technique. Spectra is generally simple, due limited fragmentation ($M+H^+$).	Difficult to quantify amount of API.
SEM/EDX	Can analyse the morphology of a tablet. Can identify and quantify inorganic components readily using EDX. Can map the distribution of elements using EDX.	Surface technique. EDX limited in that it cannot detect the lighter elements, e.g. H, He, Li and B.

The techniques that meet these requirements are Near Infrared (NIR) Spectroscopy, Raman Spectroscopy, Fourier Transform Infrared (FTIR) with Attenuated Total Reflectance (ATR) accessory, Atmospheric Solids Probe (ASAP) Mass Spectrometry, Direct Insertion Probe (DIP) Mass Spectrometry and Scanning Electron Microscopy (SEM) / Energy Dispersive X-ray Spectroscopy (EDX).

Costlier techniques such as Liquid Chromatography Tandem Mass Spectrometry (LC-MSMS) and Nuclear Magnetic Resonance (NMR), which are common in HIC, were excluded on being beyond the scope of laboratories in LMIC's. The British Pharmacopeia (BP) was used as a reference test and it should be remembered that the methodology was designed to test multiple tablets in lengthy procedures. On this basis, the BP methods do not fit the criteria for a rapid procedure and single tablet capability. The purpose of this study is to identify a rapid detection

method for substandard and falsified medicines that can be performed on a small volume of sample.

3.1 Spectroscopic Methods

3.1.1 Ultraviolet (UV) analysis

Ultraviolet (UV) and visible radiation constitute a small part of the Electromagnetic spectrum. This radiation then interacts with matter and a variety of processes can occur including absorbance, this is important for the purpose of UV-Vis spectroscopy. UV-Vis is often used for the quantification analysis of all molecules that absorb UV-Vis radiation (RSC, 2017).

The principle of UV analysis is that in the case of a single beam spectrophotometer; light is emitted from a source and is separated into narrow bands of specific wavelengths selected by a monochromator, this then passes through the sample and is measured by the detector (Harris, 2003). Depending on the spectrometer, both a tungsten and deuterium lamp can be used; this is due to the optimum performance of the lamps at a given wavelength. Tungsten lamps are often used as a continuous source of visible and Near Infrared (NIR) radiation and perform over the range of 320 – 2500nm. For lower wavelengths, a deuterium lamp is used (200 – 400nm). The source changes between deuterium and tungsten at 306nm, this is because the source with the highest intensity is always used (Harris, 2003). Once the light passes through the sample it is measured by a detector (phototube), which when struck by photons produces an electronic signal (Harris, 2003).

For the application of substandard and falsified medicines, UV analysis is often used as a confirmatory technique through comparison of an observed spectrum and a reference spectrum. In 2015, Figueroa *et al.,* exclusively used UV spectroscopy to detect substandard and falsified medicines. The group analysed adulterated samples of acetaminophen and Tylenol to establish if UV spectroscopy could identify the concentration of API in different adulterants. Results showed that at a specific wavelength the amount of change alongside characteristic shifts enabled UV to be used in the detection of substandard and falsified medicines. The absorbance of standard solutions were compared and any differences observed showed substandard nature. Shifts in the λ_{max} would indicate different species present in the solution but not identify what (Figueroa *et al.,* 2015).

Other research groups have used UV analysis in a confirmatory capacity; it has been applied to both branded and generic formulations. Baratta *et al.,* (2012) analysed 221 samples of various medications using the European Pharmacopeia (EP). The samples included antibiotics, anti-

inflammatories, antimalarials and antihypertensive, were assayed, and analysed using UV spectroscopy. (Baratta *et al.,* 2012). Vredenbregt *et al.,* (2006) used UV as a reference test to identify and quantify the API when analysing suspect Viagra samples by NIR (Vredenbregt *et al.,* 2006). Again, using UV as a reference test Tipke *et al*., (2008) analysed antimalarial drugs in Burkina Faso, using a minilab. Samples that failed this test were sent to Germany for quantification of the API by UV according to EP specifications, further analysis revealed samples to be substandard (Tipke *et al*., 2008).

3.1.2 Infrared Spectroscopy

The measurement of absorption of infrared radiation brought about by changes in molecular vibrations within molecules, gives rise to infrared spectroscopy. Depending on the wavelength of this radiation, fingerprint spectra of molecular structures can be obtained. This data provides information on the structure of the molecule and in particular the nature of the functional groups present in the sample. Raman spectroscopy provides data on similar functional groups within the molecule, the data provided is in the form of emissions at specific wavelengths. Both sets of information are produced between 4000cm^{-1} an 400cm^{-1}. The relationship of the different forms of IR analysis technique to the rest of the Electromagnetic (EM) spectrum are shown in Figure 3.2.

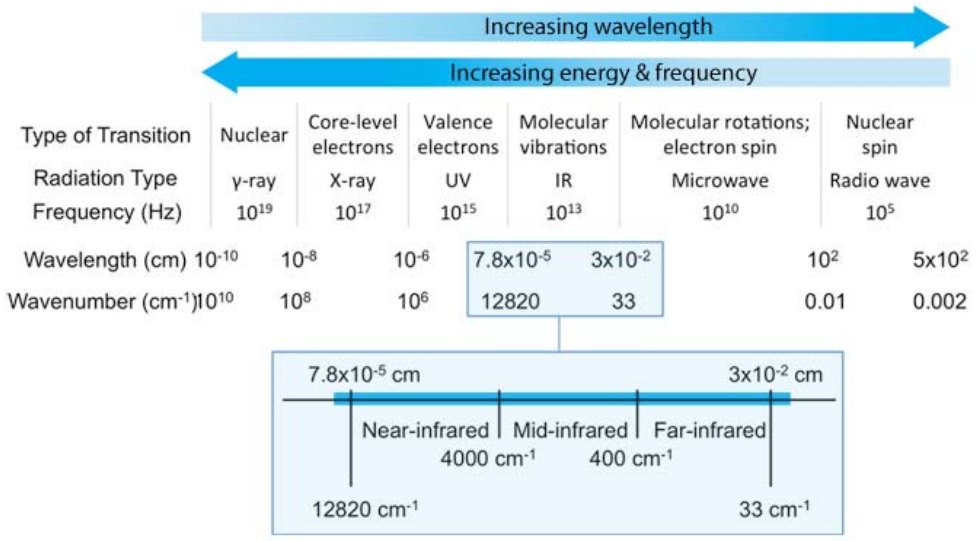

Figure 3.2: Electromagnetic spectrum and Infrared region (Chromacademy, undated)

The three different types of infrared spectroscopy have been involved in the study of SF medicines; these include NIR, ATR FTIR and Raman.

3.1.2.1 *Near Infrared (NIR) Spectroscopy (12,000 – 4000cm^{-1})*

In the past decade, Near Infrared (NIR) Spectroscopy has gained wide acceptance within the pharmaceutical industry (Reich, 2005) for various applications, including raw material testing, product quality control and process monitoring (Reich, 2005). It was once the method of choice for analysing counterfeit medicines (Lopes and Wolff, 2009).

NIR is a form of molecular spectroscopy, covering the transition from the visible spectral range to the mid infrared region. Samples are illuminated with a broad spectrum of NIR light, which can be absorbed, transmitted, reflected or scattered by the sample (Thermofisher, undated)(Figure 3.3). NIR uses overtone and recombination bands in the range of 12,000cm^{-1} to 4000cm^{-1} to determine the structure of the sample (Marks and Campbell, 2008). The most prominent absorption is related to the overtones and combinations of fundamental vibrations of –CH, –NH and –OH functional groups (Reich, 2005). The particular combinations detected are dependent on the nature of the primary light source.

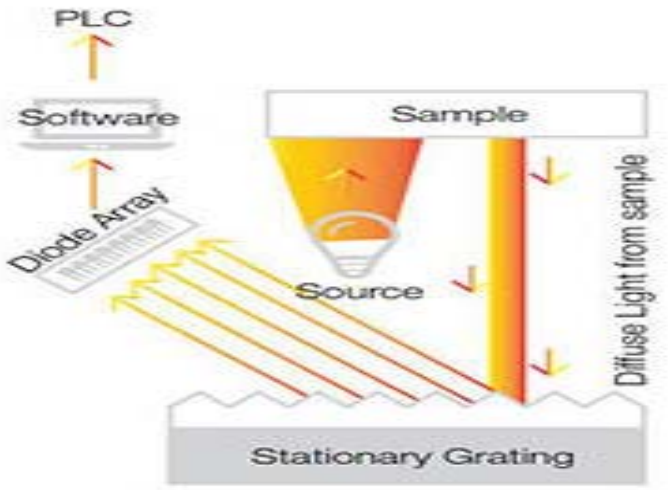

Figure 3.3: Schematic of a Near Infrared spectrometer (Buchi, 2017)

NIR provides rapid, quantitative analysis of tablets with little or no sample preparation required (Marks and Campbell, 2008; Laasonen *et al*., 2004; Baer *et al*., 2007 and Roggo *et al*., 2007). The technique is non-destructive, allowing samples to be subsequently analysed by other techniques (Roggo *et al*., 2007 and Charvill, 2005). The data produced provides molecular information not only of the API, but also of other excipients present from a single measurement (Marks and Campbell, 2008; Laasonen *et al*., 2004 and Baer *et al*., 2007). Furthermore, the cost per test for NIR is often much lower than for other analytical methods, especially when large numbers of samples need to be analysed (Marks and Campbell, 2008). These comments in general, relate to

well controlled production conditions where the direct insertion of the NIR probe into the sample is a simple QC procedure rather than analytical test of the unknown.

There are some disadvantages with NIR, only broad bands are observed and the spectra can be difficult to interpret (Roggo *et al*., 2007). The observed absorbance bands are the result of overtones from different functional groups within the sample analysed. Molecules with a similar chemical structure will therefore be difficult to distinguish using NIR. Mixture analysis will also therefore present spectra with superimposed absorptions and compounds might only be recognised if they have a unique functional group (Baer *et al*., 2007). Careful calibration and acquisition of standard spectra of active ingredients and excipients is required in order to ensure accurate quantitative identification. This is relatively easy for the pharmaceutical industry expecting repeat batches of standard production raw materials and may be possible for repeat batches of tablets. NIR spectra are sensitive to samples that absorb water from the atmosphere, it has been implied that storage conditions of samples such be considered when using NIR (de Peinder *et al*., 2008 and Moffat *et al*., 2010). Whilst NIR can be considered a rapid technique to obtain spectral data, the lack of data in a NIR spectrum combined with the complexity of the signal means that very often specialist software is required to perform the complex calculations to determine sample composition (Marks and Campbell, 2008), which can be time consuming. It may also require concentrations of each analyte to be measured by another technique (Marks and Campbell, 2008).

NIR has been used to check batch homogeneity and is able to distinguish counterfeits and imitations from authentic Viagra (de Veij *et al*., 2008). Forensically, it has been applied to identify and quantify the amount of 3,4-Methylenedioxymethamphetamine (MDMA) in ecstasy tablets, by comparing NIR spectra (Baer *et al*., 2007).

3.1.2.2 *Attenuated Total Reflectance Fourier Transform Infrared (ATR FTIR) Spectroscopy (4000 – 400cm^{-1})*

Fourier Transform Infrared (FTIR) Spectroscopy is a sophisticated tool in the spectral analysis of organic compounds (Cheng *et al*., 2010). FTIR is used to obtain structural information about a compound and has many advantages as an analytical technique. This is because all active pharmaceutical ingredients give an Infrared (IR) spectrum; the fingerprint region of the spectrum is unique and can be used for identification (Cheng *et al*., 2010). The fingerprint region is between 2000cm^{-1} and 400cm^{-1}, the vibrational frequencies and individual molecular motions observed in this range are unique to a particular molecule. Therefore, the likelihood of misidentification using FTIR is less compared to NIR techniques.

Traditional FTIR analysis of pharmaceutical samples, rely on sample extraction, solvent extraction/drying and subsequent Potassium Bromide (KBr) disc analysis. This can be difficult, as making a KBr disc is time consuming and ensuring the correct ratio of sample and KBr to provide good radiation transmission through the sample can be hard to achieve. This sample preparation approach can be replaced by modern Attenuated Total Reflectance (ATR) techniques and the increased sensitivity of FTIR spectrometers make ATR a simple and routine test (Cheng *et al*., 2010, Ortiz *et al*., 2013). ATR has the ability to measure a wide variety of solid and liquid samples without the need for complex sample preparation. ATR is achieved by IR light being totally internally reflected at the interface between the sample and the ATR crystal (Chan *et al*., 2003).

Figure 3.4 illustrates the principle of ATR. The penetration depth depends on the wavelength, the refractive indices of the ATR crystal and the sample and the angle of the beam. This typically can be between 0.5µm and 5µm (Anon, 2005). The light penetrating the sample is attenuated by interaction with characteristic molecular vibrations to produce a conventional IR spectrum (Chan *et al*., 2003). It is therefore necessary to ensure maximum surface contact between the sample and the ATR crystal. This necessity is a limiting factor of the ATR technique.

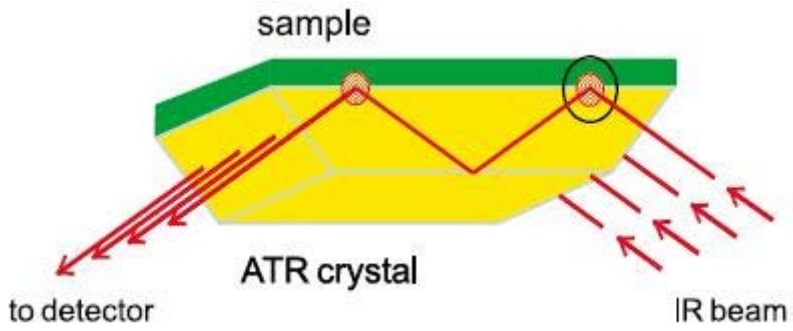

Figure 3.4: ATR principle (Tanna *et al*, 2013)

ATR FTIR is a powerful technique which has successfully been used to analyse a wide range of samples, including the polymorphic content of bulk pharmaceuticals (Salari and Young, 1998), pharmaceutical formulations (Chan *et al*., 2003), powder mixture (Planinšek *et al*., 2006) dentifrice adulteration (López-Sánchez *et al*., 2008), presence of Sildenafil and Tadalafil as adulterants in dietary ingredients (Champagne and Emmel, 2011), Chinese herbal and animal medicines (Cheng *et al*., 2010), and the origin of wines (Tarantilis *et al*., 2008). It is a fast and straightforward technique with the need for little or no sample preparation (Cheng *et al*., 2010 and Planinšek *et al*., 2006), with the ability to analyse samples in their 'natural state' with no

interference from Potassium Bromide or Nujol signals (Cheng *et al*., 2010). This technique can be classed as a rapid technique for the surface analysis of powders.

The use of ATR FTIR to quantify API's in tablet formulations in order to identify substandard or falsified medicines is relatively new. Although conventional FTIR has been applied to quantifying anti-diabetic medicines for quality control and the possible detection of counterfeits (Farouk *et al*., 2011). Lawson *et al*., (2014a) used an ATR FTIR technique to quantify the amount of paracetamol in tablets and this data was then compared to an establish BP UV methodology.
One limitation to ATR FTIR is that it does not sample the bulk of a sample; this is because IR radiation only penetrates a few microns into the sample, meaning ATR FTIR is classed as a surface technique (Bugay, 2001 and Planinšek *et al*., 2006). This may lead to misleading analysis of the whole tablets especially those that are coated. Previous analyses of some tablets have shown that whilst the stated dosage is correct the distribution of the API throughout the tablet is not uniform (Lawson *et al*., 2014b). Homogenisation of the samples especially for quantitative analysis is therefore recommended. It has been used for both medical products and medicines; in 2008, López-Sánchez used this technique to establish if toothpaste samples had been adulterated. The study was able to quantify diethylene glycol in dentifrices by using Partial Least Squares (PLS) method (López-Sánchez *et al*., 2008).

With regards to counterfeit/ substandard medicines, Been *et al*., (2011) used FTIR in combination with NIR and Raman to identify a range of medicines manufactured by F. Hoffmann and La Roche Limited and characterise the chemical composition. The study used FTIR microscopy to aid the identification and found the results to be complementary to those obtained by NIR and Raman (Been *et al*., 2011). Other studies have identified counterfeit Cialis and Viagra from uncoated milled tablets (Ortiz *et al*., 2013) and artesunate and other antimalarials (Ricci *et al*., 2007).

Salari and Young (1998) attempted to use peak height to quantify mixtures of polymorphs and found that there were limiting factors affecting the results. The most important factor was mixing efficiency; the study found that a perfect mixture was 'virtually unattainable with powders', though it was possible to achieve a mixture with a maximum degree of randomness. They also cited concerns that interfacial properties of the original material could be replaced when mixed with a lubricant, which was able to coat the powder surface (Salari and Young, 1998). SEM was used to observe the organisation of the mixture and to see if a perfect mixture could be attained. It was also found that water content influenced the agglomeration of particles (Salari and Young, 1998).

Much of the work reported above is qualitative in nature and as all spectroscopic signals depend on the concentration of the analyte in the sample, the original weight of any tablet must be determined before quantitative procedure can be carried out.

3.1.2.3 *Raman Spectroscopy*

Until the late 1990's, Raman spectroscopy was not widely applied in the pharmaceutical industry; this was due to the industry being less sure of the quality of its raw materials and therefore the need to be able to perform rapid QC checks on the materials. Smaller detectors that are more efficient coupled with small efficient lasers, as the radiation source have led to the development of portable handheld Raman instruments. (Webster and Baldwin, 2005).

The use of lasers, particularly in handheld systems introduces the problems of radiation safety protection. This is of importance where direct sampling of tablets is concerned. In this situation, the radiation extends beyond the body of the instrumentation. Several approaches to protecting the individual have been used:

a) Enclosed tablet sample holder, which attaches onto the instrument.
b) Sample proximity devices limiting the use of the laser.
c) Specially constructed surround shields.

This concern produced a delay in ~18 months in accessing and the availability of equipment at DMU.

Similar to other IR absorption techniques, Raman spectroscopy is a form of vibrational spectroscopy (Webster and Baldwin, 2005) and is closely related to near- and mid- IR absorption spectroscopy (Bugay, 2001). It has been described as a complementary technique to IR (Pivonka *et al.*, 2007; Bumbrah and Sharma, 2016). Whilst some molecular vibrations may be observed in both Raman and IR spectra, often vibrations observed using one technique would be extremely weak or absent in the other (Webster and Baldwin, 2005). For example, water has strong IR absorptions, but is a weak Raman scatterer; therefore, enabling aqueous solutions to be studied using Raman, as the signal of the sample is not swamped by that of the solvent (Banwell and McCash, 1994).

When a beam of monochromatic (laser) radiation passes into a sample (Figure 3.5), it will interact with the molecules within the sample; the radiation may be reflected, absorbed or scattered (Horiba, 2017). The frequency of the scattered radiation will consist of both incident radiation (Rayleigh scattering) and a small amount of scattered light at a different wavelength (Stokes and

Anti-Stokes Raman scattering). Only a small fraction of the scattered light constitutes of Raman scattering (~1 x 10^{-7}) (Horiba, 2017).

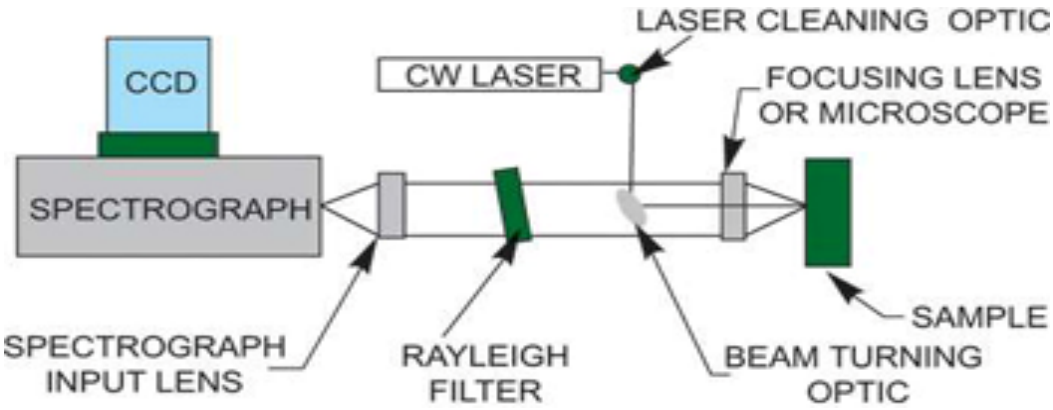

Figure 3.5: Schematic of a Raman spectrometer (Andor, 2017)

Rayleigh scattering occurs when there is no change in the frequency of the scattering process. If a change in frequency is observed then this process is Raman scattering, this process can determine structural and chemical information (Horiba, 2017). Depending on the vibrational state of the molecule, Raman shifted protons can be either lower (Stokes) or higher energy (Anti-Stokes).

Stokes scattering is the stronger of the two processes and occurs when a photon is scattered at lower energy, it is stronger as, principally at room temperature molecules are in their ground vibrational state (Horiba, 2017). The weaker Anti-Stokes Raman scattering occurs when the scattered photon can be scattered at a higher energy. This happens when a molecule is at a higher vibrational energy level; only a few molecules are ever in this state (Horiba, 2017) (Figure 3.6).

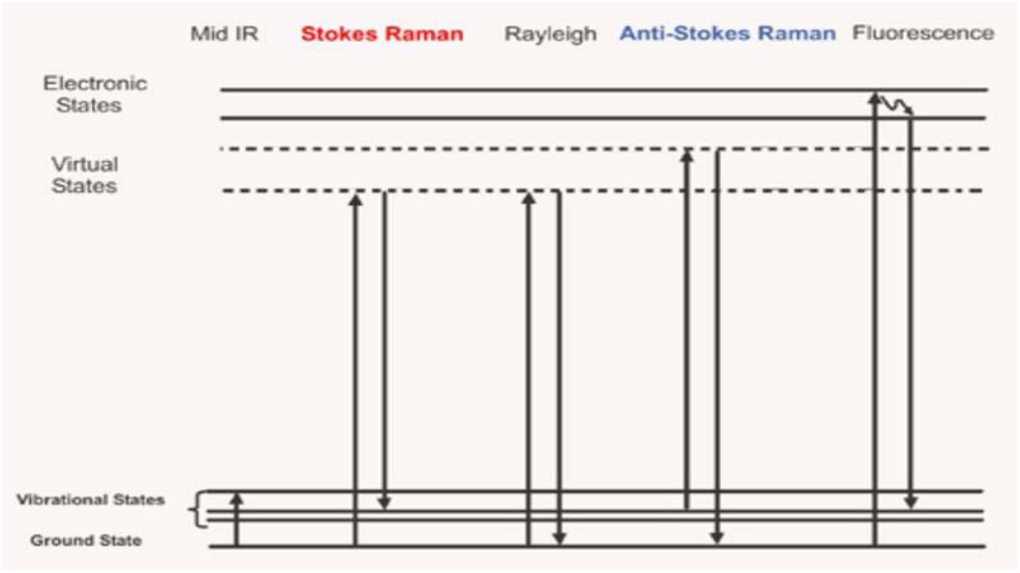

Figure 3.6: Jablonski Diagram (Horiba, 2017)

Raman spectroscopy enables the molecular structure of a sample to be characterised, as the interaction of the incident photon with the molecule and the energy change of the proton is characteristic of the bond (vibration) present. It is important to note that not all vibrations are observable with Raman spectroscopy (Horiba, 2017).

An essential requirement of obtaining a Raman spectrum of a sample is that a change in polarisation during molecular vibration must be observed. A typical Raman spectrum plots intensity versus wavelength shift and is measured over a range of 4000-100cm^{-1} (Bumbrah and Sharma, 2016). Features in Raman spectrum corresponding to the various vibrational modes of a molecule are generally sharp and well resolved for many API's (Webster and Baldwin, 2005).

Raman also shares many of the advantages of NIR spectroscopy, in that it is a form of non-destructive analysis and can analyse both tablets though outer coatings (de Veij et al., 2008) and samples through glass (Bugay, 2001). Both techniques have been proposed as a fast-reliable analytical method, as there is minimal or no sample preparation.

Uncertainty about reproducible penetration depth and subsequent emissions for comparable samples has led to the need to use Principal Component Analysis (PCA) techniques for identification of the major API in tablets by the manufacturers of some handheld instruments.

The primary advantage of Raman over NIR, is that Raman spectra show highly specific spectral fingerprints (Marks and Campbell, 2008) and can be used for polymorph identification (de Veij *et al*., 2008). Figure 3.7 and Figure 3.8 show the differences between the two spectra for both an inorganic compound (Figure 3.7) and an organic compound that fluoresced (Figure 3.8). Even

when a sample fluoresces, Raman spectra yield a more detailed fingerprint than that of a NIR spectrum.

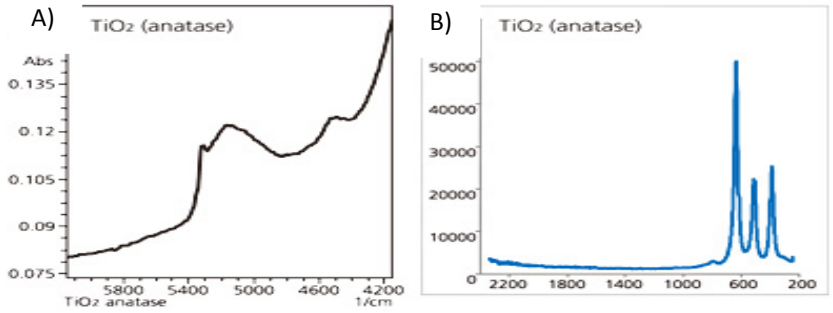

Figure 3.7: Analysis of TiO_2 anatase, a) NIR Spectroscopy; b) Raman Spectroscopy (Shimadzu, 2016)

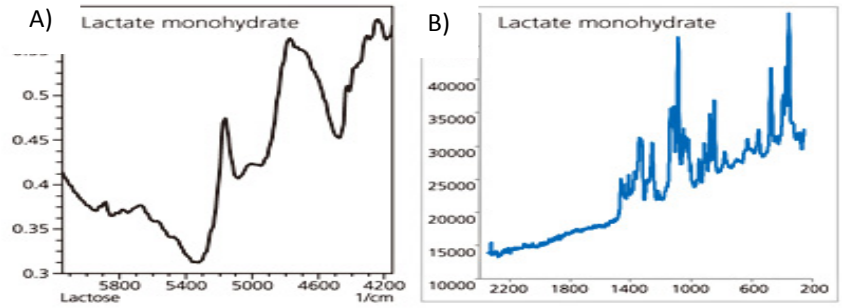

Figure 3.8: Analysis of lactose monohydrate, a) NIR Spectroscopy; b) Raman Spectroscopy (Shimadzu, 2016)

A limitation to this technique relates in particular to coloured samples, it has been found that heat generated by the focused laser beam can cause decomposition of the sample. Another significant limitation is sample fluorescence (Figure 3.6), which results from excitation to a higher electronic state and subsequent return to the ground state. Samples of an 'organic and biological' nature often fluoresce, the amount and type of fluorescence is dependent on the material analysed and laser wavelength (Renishaw, 2015). For example, exciting this type of samples with a laser with the wavelength of 532nm, could promote fluorescence therefore swamping any underlying Raman spectrum and masking the signal (Horiba, 2017). If a laser with a longer wavelength, particularly one in the NIR region (785nm), the effect of fluorescence is often reduced, as the electronic transition is not promoted, allowing the Raman signal to be easier to detect. However, by increasing the wavelength, the scattering efficiency decreases, therefore requiring a higher laser power or longer integration times (Horiba, 2017).

Raman spectroscopy is widely used in the pharmaceutical industry, for monitoring and studying API's, quantitative studies and real-time release testing (Farcaş *et al.*, 2016 and Esmonde-White *et al*., 2017). It is often the technique of choice when identifying counterfeit medicines using both

benchtop and handheld instruments, Raman spectroscopy has been used to identify both lifestyle (Viagra) (de Veij *et al*., 2008) and live saving medicines (antimalarials) (Bate and Hess, 2010; Kwok and Taylor, 2012 and de Veij *et al*., 2007)). It has also been used for the authentication of counterfeit branded and generic medicines (Assi, 2016 and Kwok and Taylor, 2012) and the in-situ identification of drugs of abuse in an airport environment (Hargreaves *et al*., 2008). Due to occurrences of falsified and substandard medicines rising significantly, the need for rapid identification of these medicines is critical (Dégardin *et al.*, 2017). The quality of medicines reaching the patient in some countries can vary enormously, with substandard products changing to respond to better detection capabilities (Bate and Hess, 2010). This insidious risk can have an impact on the patient, and are likely not to cure the condition and put the individual at risk (Bate and Hess, 2010 and Dégardin *et al.*, 2017).

The advent of the handheld Raman spectrometer, has enabled the lab to be brought to the samples, numerous studies have analysed counterfeit medicines using handheld spectrometers. Ricci *et al*., (2008) analysed counterfeit artesunate tablets, they observed the effects of fluorescence when using a handheld spectrometer, but used this to their advantage as the API (artesunic acid) fluoresced; tablets that did not fluoresce confirmed the absence of the API (Ricci *et al*., 2008). Bate and Hess (2010) compared the handheld Raman spectrometer to the Minilab system that was already used in the field. The study analysed antimalarials and found that Raman spectroscopy could generate a unique fingerprint signal that reflected the content of the whole sample. Concerns over counterfeiters adapting their actions to fool the more basic minilab API assay, whilst failing to produce a quality product were discussed (Bate and Hess, 2010). Assi, (2015a) analysed a range of antierectile dysfunction samples using a dual wavelength handheld spectrometer to identify the presence of various API's. It was found by using another wavelength (1064nm), the effect of fluorescence could be mitigated and Assi was able to identify API's in the concentration of 5 to 15% of the tablet (Assi, 2015a). Identification of tablets through packaging has been successfully demonstrated (Eliasson and Matousek, 2007) and is becoming commonplace for equipment equipped with PCA analysis. Quantitative investigations in this mode can be widely erroneous if the tablet weight is unknown.

3.2 Chromatographic Methods

3.2.1 GPHF-Minilab

The Global Pharma Health Fund (GPHF) developed the GPHF-Minilab, as a basic drug testing scheme to identify counterfeit medicines. The minilab comprises of a three-stage test, these are:

- Physical inspection – enables early rejection of crude counterfeits,
- Disintegration test – verification of label claims on modified release and enteric-coated formulations,
- TLC – to check API content against labelled claims (GPHF, undated).

The visual inspection of physical attributes of the tablet is performed first in order to reject crude falsified samples. The tablet, labelling and packaging are compared with a genuine sample to establish if a sample is fake (Sherma, 2007). Samples that pass this test are then investigated by ensuring that the samples disintegrate within 30 minutes, unless the sample is modified release or enteric-coated. The final test is TLC and the Rf values for the sample are compared to a reference (Sherma, 2007). The minilab contains secondary standards for 85 different API's and has been used to identify counterfeit antimalarials, antituberculosis and antiretrovirals (GPHF, undated).

The minilab has been used in various countries, studies in Malawi by Khuluza *et al*., (2016) analysed the antimalarial sulphadoxine/ pyrimethamine. Out of the 28 samples analysed, 10 samples were identified as requiring further analysis. Out of these ten samples, one sample was identified as being falsified by the minilab which was confirmed by HPLC analysis and a further two were identified as substandard by HPLC analysis (Khuluza et al., 2016). It is important to note that the Minilab will not identify or quantify other components in the tablet, other than the target API (Sherma, 2007).

3.3 Mass Spectrometry

Mass spectrometer (MS) instrumentation is changing dramatically with most modern instruments being benchtop and there are several examples of portable MS instruments. The large magnetic sector analysers have been replaced by small quadrupole based systems, which rely on path stability criterion to give mass (m/z) analysis. One of the more significant developments has been the change from electron impact (EI) ionisation to the much less energetic proton transfer methodologies. Electron impact ionisation is conducted under vacuum conditions inside the MS system, whereas proton transfer occurs at atmospheric pressure outside the vacuum system.

The species detected in each system will therefore be different: electron impact would yield molecular and fragment ions, proton transfer would yield ions at m/z value of the molecular weight plus a proton, for example $(M+H)^+$. Nonetheless, each technique provides its own reproducible fingerprint spectrum of a particular compound. The combination of the stated API with the expected mass spectrum from a database will confirm the presence or absence of the stated material.

3.3.1 Direct Insertion Probe (DIP) Mass Spectrometry (MS)

In the Direct Insertion Probe (DIP) MS system the sample, in a small vial on the end of a probe, is introduced directly into the ion source of the MS under vacuum conditions as shown in Figure 3.9. The sample is rapidly heated under controlled conditions (up to 500^0C), species released from the powder sample are ionised in the electron beam (20 - 70eV), and the ions are directed into the MS for analysis. Due to the high ionisation energy, some fragmentation of the molecular ion may be observed.

This technique has been used in a range of applications, such as polymer science (Hacaloglu, 2012), investigation of low-level pollutants in water (Bauer and Cooks, 1993), for the determination of organic compounds in aqueous samples (Bier *et al.*, 1990) and to investigate conductive and non-conductive materials. Modern DIP MS technology has yet to be applied to the investigation of counterfeit medicines.

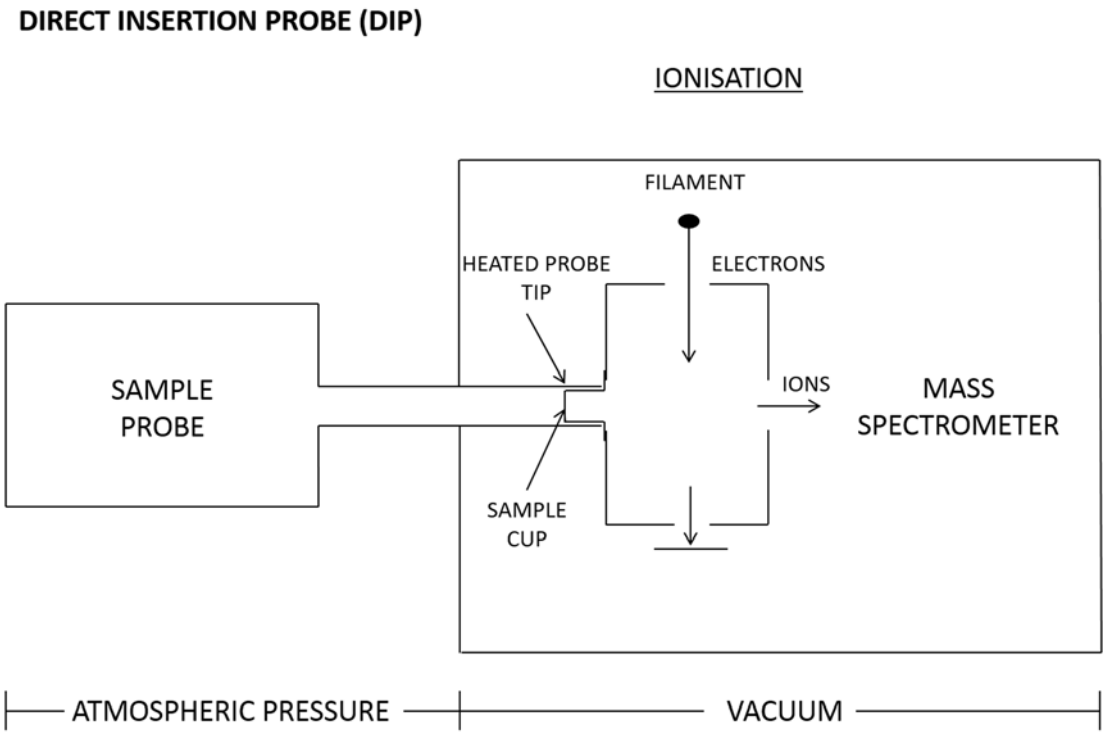

Figure 3.9: Schematic of a DIP system

DIP MS is a rapid and cost-effective technique, as it needs little sample preparation and components are separated as a function of their volatilities and/or thermal stabilities (Hacaloglu,

2012). Depending on the conditions used, this process can be repeated once every few minutes, allowing for a high throughput of samples.

The use of direct insertion probe MS analysis of API's in fully formulated tablet forms to identify substandard and falsified medicines is novel.

3.3.2 Atmospheric Solid Analysis Probe (ASAP) Mass Spectrometry (MS)

The use of the Atmospheric Solid Analysis Probe (ASAP) MS system involves a glass capillary, containing the powdered sample, being inserted into a heated stream of nitrogen gas (100-500^{0}C), which vaporises the sample (Twohig *et al*., 2010). The vaporised sample is ionised by a corona discharge and ions are then focussed directly into the mass spectrometer (Lawson *et al*., 2016). Figure 3.10 shows the schematic of ASAP MS systems. The sample is not introduced into the vacuum regions.

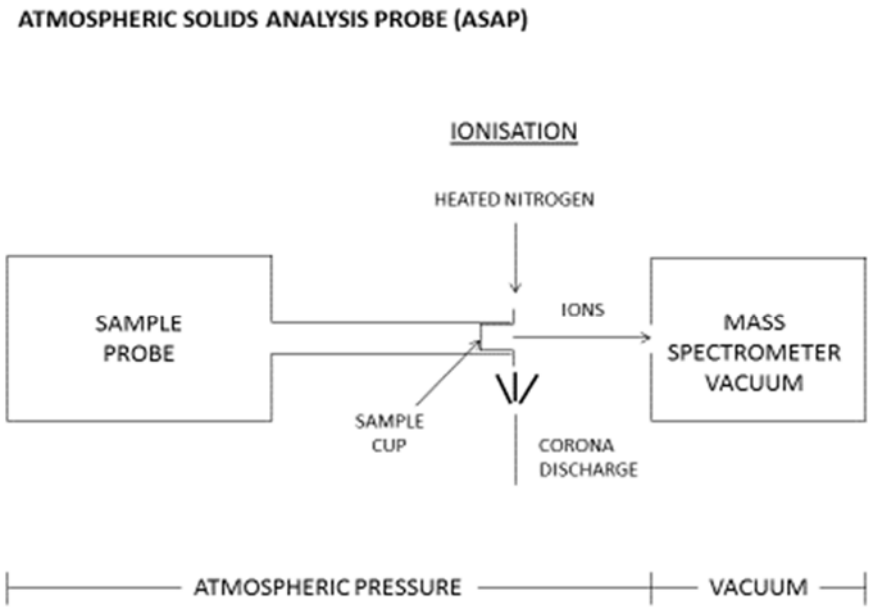

Figure 3.10: Schematic of an ASAP system

The resulting spectra are very simple, as the low energy of the corona discharge (12.6eV) produces little fragmentation (Lawson *et al*., 2016).

The overall analysis time of the samples is significantly reduced when compared to other MS techniques, due to the elimination of chromatographic separation (Twohig *et al*., 2010). Another

major advantage to this technique is that since the sample preparation requires only crushing and the spectra generated are simple, thus limiting the need for specialist operators.

ASAP MS has been used to identify counterfeit synthetic phosphodiesterase type 5 inhibitors. The study by Twohig *et al*., (2010) effectively used this technique to rapidly detect unknown compounds (Twohig *et al*., 2010). ASAP MS is a rapid technique that can be used to identify counterfeit medicines (Twohig *et al*., 2010). This technique can be used to analyse both volatile and semi volatile solid or liquid samples (Twohig *et al*., 2010).

Lawson *et al*., (2016) have also reported the use of ASAP MS to identify falsified and substandard products. Their research focused on antimalarials, atenolol, aspirin, caffeine and paracetamol and compared ASAP MS to another MS technique. The study found that API's could be readily identified due to the simplicity of the spectra.

3.4 Electron Microscopy

3.4.1 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) has been widely used in both the pharmaceutical and forensic industries for the characterisation of API's, excipients and formulated products (Nichols, 2012). This technique can be coupled to an Energy Dispersive X-ray Spectroscopy (EDX) detector, which enables elemental analysis to be performed.

The ability to use SEM/EDX to visualise rapidly the spatial distribution of particles in a solid dosage form is a desirable as a method to support development and manufacture of medicinal products (Nichols, 2012). However, there is a limitation to using EDX mapping, if the elements occurring in the sample are lighter than beryllium, then the drug may not be distinguishable from the excipients. Lithium used for the treatment of bipolar disorders is an example of this situation. When the species being sought contains 'heavy atoms', then mapping its distribution can be achieved by EDX (Kotula *et al*., 2003). It is important to note that SEM/EDX is a surface technique.

SEM utilises the analysis of different emissions when a high-energy (1kV – 30kV) beam of electrons impacts on the surface of the sample. Figure 3.11 identifies the most frequently studied emissions from the surface:

- Secondary Electron (SE) – information relating to surface structure (topographical)
- Backscatter (BSE) – information relating to composition and topography
- X-ray – information relating to elemental analysis
- Cathodoluminescence – information relating to the chemistry and structure

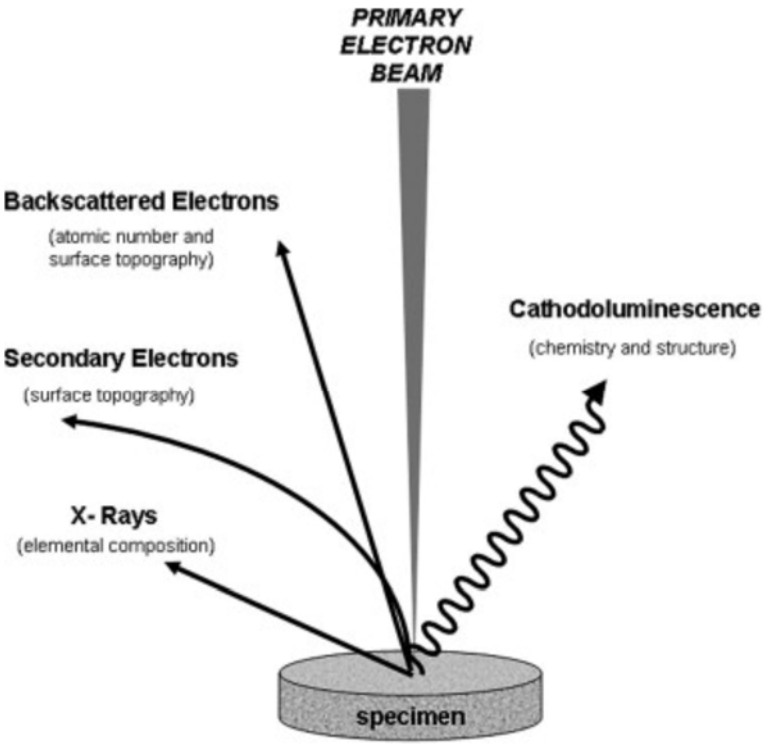

Figure 3.11: Schematic diagram showing some of the signals that are generated when a primary electron beam interacts with a specimen in the Scanning Electron Microscope (Nichols, 2012)

These methods only provide molecular information, whereas Cathodoluminescence (CL) can provide chemical information. This technique has recently been exploited for the analysis of organic compounds and pharmaceutical materials (Nichols, 2012).

CL is most commonly used in geological studies (Götze *et al*., 2001; Seyedolali *et al*., 1997) and material sciences (Kwon and Boggs, 2002; Rosner and Girolami, 1999). However, it is now being applied to pharmaceutical materials (Nichols, 2012). CL is defined as the production of electromagnetic radiation (Ultra Violet, Visible and Near Infrared), as a result of an internal chemical reaction (Mestre *et al*., 2001). The emission of light resulting from this reaction is when one product returns to its ground state from its excited state (Mestre *et al*., 2001). Spectral analysis of the emitted CL radiation reveals characteristic spectra for pharmaceutical active ingredient compounds. Panchromatic CL information can be generated using a VPSE detector with the collector bias set to 0.

SEM has been used extensively for morphological and characterisation studies. Hendrick *et al*., (2010) used SEM to determine if nanoparticles had been incorporated into a matrix and to determine if electrospun fabrics had consistent morphology (Hendrick *et al*., 2010). The technique has also been applied to the investigation of the interactions between chitosan and ibuprofen and to assess the morphology of the nanocomplex formed (Abioye *et al.,* 2016). SEM/ EDX has been used for qualitative and quantitative analyses of drug impurities in drug substances and drug

products, and focused on confirming the presence of a fumarate salt (Berridge, 1995). Sousa *et al.* (2008) used SEM/ EDX to analyse the formation of an apatite layer of Calcium Phosphate with an API (atenolol). Forensically Vogt and Neilly (2010) employed SEM/ EDX to characterise two tablet formulations; it was found that one tablet contained particles of stainless steel and the other was organic with inorganic formulations. SEM was used to confirm the logo dimensions, whilst EDX determined the elemental composition, which was compared to the chemical composition identified by FTIR (Vogt and Neilly, 2010).

There is an absence of published literature for this technique when applied to the detection of SF medicines. Microscopy has been applied to the area of counterfeits, in particular Raman and FTIR microscopy. Adar *et al*. (2014) compared both tablet forms and packaging to identify counterfeits using Raman microscopy (Adar *et al.,* 2014). A study by Lanzarotta *et al*. (2011), identified counterfeit tablet cores using FTIR microscopy, the study was part of the FDA's multidisciplinary approach, this includes physical, chromatographic and mass spectromic, elemental and molecular information being utilised (Lanzarotta *et al*., 2011). The group considered the effect of excipients and the excipient profile on the efficacy of the API. It has been previously found that the presence of incorrect excipients and/ or the absence of correct excipients can create issues with the API. This can ultimately result in the potential for more frequent adverse patient reactions; this is mainly due to higher impurity levels and faster API degradation (Lanzorotta *et al*., 2011).

Whilst vibrational spectroscopy continues to expand for the investigation of counterfeits (Lanzarotta *et al.,* 2011), very few studies have investigated the elemental composition. There is also an absence of studies investigating molecular information, using CL to analyse pharmaceutical materials (Nichols, 2012).

This current research focuses on the EDX mapping and quantification of cross-sectioned tablets and investigates its potential for the detection of SF medicines and looks at deconstructing the formulation of the tablet.

3.5 Comparison of Techniques

The application of the identification of falsified and substandard medicines applicable to LMIC requires consideration of more than just the capabilities of the instruments. Limited infrastructure in rural areas or portability requirements may define which instruments are suitable. Cost of the basic equipment and necessary consumables will also be the overriding concern in the decision-making process.

Whilst all the instruments discussed here can be applied in a 'portable' format, it is probably correct to say that only Raman systems can be described as giving handheld analyses.

Table 3.2 compares some of the parameters of the techniques used in this work.

3.5.1 General reliability of methods

The general reliability of the analytical techniques used in this research are discussed below, the robustness of these methods will be discussed in section 7.5.

3.5.1.1 *UV Spectroscopy*

Non-specific quantification technique, which when used in conjunction with other separating techniques has been demonstrated to produce validated quantification data for API's, as evidenced by the British Pharmacopeia (British Pharmacopeia 2017b).

3.5.1.2 *Infrared Spectroscopy*

Specificity with respect to the identification of individual compounds, which is comparable to a fingerprint and is another basic tool cited by the British Pharmacopeia.

3.5.1.3 *Raman Spectroscopy*

Like Infrared, Raman demonstrates specificity with respect to the identification of individual formulations, which is comparable to a fingerprint for a tablet formulation.

3.5.1.4 *ASAP Mass Spectrometry*

Atmospheric ionisation that produces ions of a charge to mass ratio (m/z) values characteristic of the target API.

3.5.1.5 *DIP Mass Spectrometry*

Conventional Mass Spectrometry produces fragmentation of the target molecule, which is accepted globally as an identification method via the National Institute of Standards and Technology (NIST) database.

3.5.1.6 *EDX Spectroscopy*

When a high-energy electron beam interacts with the surface of a sample excess energy is released in the form of electromagnetic radiation, in this case X-rays. The energy of radiation (X-rays) uniquely identifies the element from which it came from.

Table 3.2: Comparison of various analytical techniques

Technique		**Sample**	**Technique**	**Sampling Depth**	**Limit of Detection**	**Analysis Time**	**Sampling Probe/ eV**
UV	Identification						UV/ 2 - 5
	Quantification	Extract API	Fixed Wavelength	Bulk	<0.1% of tablet weight	>30 minutes	
ATR FTIR	Identification	Tablet/ Powder	Fingerprint	Surface		~2 minutes	IR/ 0.1 – 0.8
	Quantification	Powder	Peak Area	Surface	5% of tablet weight	< 5 minutes	
Raman	Identification	Tablet/ Powder	Fingerprint	Sample dependent		~2 minutes	Laser/ 1.9 or 1.6
	Quantification	Powder	Peak Area	Sample dependent	3% of tablet weight	< 5 minutes	
DIP MS	Identification	Powder	m/z value	Bulk		10 – 15 minutes	Volatilisation and Electron Beam/ 20 – 70
	Quantification	Powder	m/z value	Bulk	1% of tablet weight	10 – 15 minutes	
ASAP MS	Identification	Powder	m/z value	Bulk		~ 5 minutes	Volatilisation and Proton Transfer/ <16
	Quantification	Powder	m/z value	Bulk	1% of tablet weight	~ 5 minutes	
SEM/EDX	Identification	Tablet/ Powder		Surface		~15 minutes	Electron Beam/ 1k – 30k
	Quantification	Tablet/ Powder	Elemental	Surface	Atomic %	~25 minutes	

4 Instruments, Materials and Experimental Methods

This chapter presents details of the instruments and methods used to identify potential rapid screening methodologies to identify SF medicines.

The instrumental methods used in this study to identify the API were:

- ATR FTIR Spectroscopy,
- Raman Spectroscopy,
- Direct insertion probe (DIP) Mass Spectrometry
- Atmospheric sampling probe (ASAP) Mass Spectrometry.

The instrumental methods used in this study to quantify the levels of the API were:

- UV Spectroscopy,
- ATR FTIR Spectroscopy,
- EDX Spectroscopy

Chapter 5 discusses the results obtained from the experimental work for the identification of the API in both a whole and crushed tablet sample.

4.1 Instrumentation

The instruments used for this research are detailed below:

- UV Spectroscopy
    - A Helios Gamma UV-Vis spectrometer (Thermo Electron Corporation, UK), was used to measure the absorbance of the samples at a fixed wavelength measurement at 275nm (atenolol) and 232nm (metformin hydrochloride).
- ATR FTIR Spectroscopy
    - An Alpha with ATR accessory FTIR spectrometer, (Bruker, Coventry, UK), was used to acquire data at a spectral resolution of $2cm^{-1}$ over a scan range of $4000cm^{-1}$ to $400cm^{-1}$. Spectral data was acquired with OPUS software, version 7.5 (Bruker, Coventry, UK).
- Raman Spectroscopy
    - A Bravo handheld Raman spectrometer, (Bruker, Coventry, UK) fitted with a bag tip holder was used to acquire data at a spectral resolution of $12cm^{-1}$ over a scan range of $3200cm^{-1}$ to $300cm^{-1}$, using laser excitation of 785nm and 1084nm. Spectral data was acquired with OPUS software, version 7.5 (Bruker, Coventry, UK).

- A MIRA-3 handheld Raman spectrometer, (Metrohm, Runcorn, UK) fitted with a curvette sample holder was used to acquire data at a spectral resolution of 14 - 16cm^{-1} over a scan range of 2300cm^{-1} to 400cm^{-1}, using laser excitation of 785nm. Spectral data was acquired with MiraCal, version 3.0.1 software (Metrohm, Runcorn, UK).
- A FORAM-2 benchtop Raman spectrometer, (Foster and Freeman, Evesham, UK) was used to acquire data at a spectral resolution of 6cm^{-1} over a scan range of 3200cm^{-1} to 200cm^{-1}, using laser excitation of 785nm. Spectral data was acquired with FORAM 3 software (Metrohm, Runcorn, UK).

- DIP Mass Spectrometry
  - A 300 single quadrupole mass spectrometer, (Bruker, Coventry, UK) equipped with a Direct Insertion Probe (DIP) was used to acquire data at a resolution of 0.5m/z over a scan range of m/z 100 to m/z 300 (atenolol) and m/z 300 to m/z 550 (antimalarials). Spectral data was acquired with Workstation software, version 7 (Bruker, Coventry, UK).
- ASAP Mass Spectrometry
  - An Advion expression compact mass spectrometer (CMS), (Advion Ltd, UK) equipped with an Atmospheric Solids Analysis Probe (ASAP) was used to acquire data at a resolution of 0.5m/z over a scan range of m/z 100 to m/z 550. Spectral data was acquired with Advion integrated software.
- SEM/EDX Electron Microscopy
  - An EVO LS 15 Scanning Electron Microscope (Carl Zeiss Ltd, Cambridge, UK) was used as a source of primary electrons for Secondary Electron (SE), Backscatter (BSE) and EDX Spectroscopy. Images were acquired using Smart SEM version 5.7 software (Carl Zeiss Ltd, Cambridge, UK). An Oxford Xmax 80 mm^2 EDX detector (Oxford Instruments, High Wycombe, UK) was used for elemental analysis. Elemental data was processed using INCA software (Oxford Instruments, High Wycombe, UK).

4.2 Materials

4.2.1 Excipient and Reference Samples

Excipient samples were selected upon the most commonly used excipients in the manufacture of tablets, as detailed in Chapter 2.2. The excipients were purchased from a variety of suppliers and are summarised in Table 4.1. There were two distinct sets of reference material used in this study:

a) the conventional analytically pure materials, b) formulated tablets as supplied to healthcare providers. The reference standards purchased for this study are detailed in Table 4.2. The solvents used for UV analysis are listed in Table 4.3.

Table 4.1: Manufacturer and batch numbers of excipients used

Excipient	**Manufacturer**	**Batch Number**
Calcium carbonate	Acros Organics (Loughborough, UK)	A0327003
Calcium phosphate	Pfizer (UK)	98EXC03
Carnauba wax	Baer Locher (France)	185598
Citric acid	JM Loveridge (Southampton, UK)	K645
Croscarmellose sodium	Sigma Aldrich (Gillingham, UK)	015K0176
Colloidal silicon dioxide	Evonik (Manchester, UK)	155070314
HPMC	Colorcon (Kent, UK)	P180F44003
Iron Oxide	Acros Organics (Loughborough, UK)	
Lactose monohydrate	Blackburn Distribution (Nelson, UK)	15058
Lactose anhydrous	Fluka (Gillingham, UK)	BCBM7015V
Lactose (Tablettose)	Meggle (Germany)	0248
Magnesium stearate	JM Brown (UK)	231106
MCC (Avicel PH101)	Fluka (Gillingham, UK)	BCBN7864V
PEG	Fisher Scientific (Loughborough, UK)	0304382
Povidone	Acros Organics (Loughborough, UK)	A0336639
SLS	Fisher Scientific (Loughborough, UK)	1484584
Sodium starch glycolate	JRS Pharma LP (USA)	E0173
Starch – maize	Fisher Scientific (Loughborough, UK)	
Starch - potato	JM Loveridge (Southampton, UK)	K714
Stearic acid	Acros Organics (Loughborough, UK)	A0327293
Talc	VWR International (Lutterworth, UK)	160180006
Tartaric acid	Sigma Aldrich (Gillingham, UK)	STBC1798V
Titanium dioxide	Sigma Aldrich (Gillingham, UK)	SZB90960V

Table 4.2: Manufacturer and batch numbers of analytical reference APIs used

API	Manufacturer	Batch Number
Atenolol	Sigma Aldrich (Gillingham, UK)	BCBL0559V
Metformin hydrochloride	Sigma Aldrich (Gillingham, UK)	LRAA8975
Chloroquine diphosphate salt	Sigma Aldrich (Gillingham, UK)	BCBM9716V
Quinine 99% anhydrous	Acros Organics (Loughborough, UK)	A0316466
Quinine monohydrochloride	Sigma Aldrich (Gillingham, UK)	STBD7305W
Quinine sulphate	Fissons (UK)	39673025

Table 4.3: Manufacturer and batch numbers of solvents used

Solvent	Manufacturer	Batch Number
Methanol (HPLC grade)	VWR International (Lutterworth, UK)	161284013
Water (HPLC grade)	Fisher Scientific (Loughborough, UK)	1367414
Isopropanol	Fisher Scientific (Loughborough, UK)	16J054025

4.2.2 Tablet Samples

Patients can source medicines by various methods. Traditionally, patients in the UK would purchase medicines over the counter (OTC) directly from a pharmacy/ supermarket, or if they required a prescription only medicine (POM), this would be prescribed by a doctor and dispensed by a pharmacist. However, with the advent of the internet, medicines can be directly purchased online. In other countries both POM and OTC medicines, can be purchased from pharmacies, the internet and from 'non-pharmacy' vendors, as is the case in Kenya and most LIC. Medicines can also be purchased without a prescription for POM's in other countries, as a personal supply to an individual. The atenolol, metformin hydrochloride and antimalarial tablets studied in this research were supplied by individuals who obtained the medicine as a personal supply from pharmacies. An antimalarial sample was obtained from an unlicensed vendor 'tuck shop'. The UK tablets were supplied by a pharmacy for research purposes.

The atenolol tablets that were investigated were obtained from five countries; these were the UK, India, Pakistan, Saudi Arabia, and Nepal. The tablets analysed are summarised in Table 4.4 and have been anonymised.

The metformin hydrochloride tablets that were investigated were obtained from three countries; these were the UK, India and Saudi Arabia. The tablets analysed are summarised in Table 4.5 and have been anonymised.

The antimalarial tablets that were investigated were obtained from five countries; these were the UK, Ghana, Zimbabwe, Nigeria and Nepal. The tablets analysed and their API's are summarised in Table 4.6 and have been anonymised.

Table 4.4: Atenolol tablet samples analysed

Name of Tablet	Dosage	Country of Origin	Number of Tablets	Location of Pharmacy
At/UK/1	25mg	UK	28	Leicester
At/UK/2	50mg	UK	28	Leicester
At/UK/3	100mg	UK	28	Leicester
At/UK/4	25mg	UK	28	Leicester
At/UK/5	50mg	UK	28	Leicester
At/UK/6	100mg	UK	28	Leicester
At/UK/7	50mg	UK	28	Leicester
At/IND/1	25mg	India	14	Gauribidnur
At/IND/2	25mg	India	14	Gauribidnur
At/IND/3	25mg	India	2	Mumbai
At/IND/4	25mg	India	2	Mumbai
At/IND/5	50mg	India	14	Darjeeling
At/IND/6	50mg	India	14	Darjeeling
At/IND/7	50mg	India	14	Gauribidnur
At/IND/8	50mg	India	14	Pondicherry
At/IND/9	50mg	India	14	Gauribidnur
At/IND/10	50mg	India	14	Gauribidnur
At/IND/11	25mg	India	14	Gauribidnur
At/IND/12	50mg	India	14	Pondicherry
At/IND/13	50mg	India	14	Gauribidnur
At/IND/14	50mg	India	14	Darjeeling
At/IND/15	50mg	India	14	Gauribidnur
At/IND/16	50mg	India	14	Pondicherry
At/IND/17	25mg	India	2	Goa
At/IND/18	50mg	India	14	Pondicherry
At/PAK/1	50mg	Pakistan	20	Lahore
At/PAK/2	100mg	Pakistan	20	Lahore
At/SA/1	50mg	Saudi Arabia	28	Mecca
At/SA/2	50mg	Saudi Arabia	28	Mecca
At/SA/3	100mg	Saudi Arabia	14	Medina
At/SA/4	100mg	Saudi Arabia	14	Medina
At/SA/5	100mg	Saudi Arabia	14	Medina
At/NEP/1	50mg	Nepal	14	Pokhara
At/NEP/2	50mg	Nepal	14	Pokhara
At/NEP/3	50mg	Nepal	14	Pokhara

Table 4.5: Metformin Hydrochloride tablet samples analysed

Name of Tablet	Dosage	Multiple API	Country of Origin	Number of Tablets	Location of Pharmacy
Met/UK/1	500mg		UK	56	Leicester
Met/UK/2	850mg		UK	56	Leicester
Met/IND/1	250mg		India	10	Gauribidnur
Met/IND/2	500mg		India	10	Gauribidnur
Met/IND/3	850mg		India	10	Gauribidnur
Met/IND/4 *	500mg		India	20	Gauribidnur
Met/IND/5 *	850mg		India	10	Gauribidnur
Met/IND/6 *	500mg	Yes	India	15	Gauribidnur
Met/IND/7	500mg	Yes	India	10	Gauribidnur
Met/IND/8	500mg	Yes	India	10	Gauribidnur
Met/IND/9	500mg		India	10	Gauribidnur
Met/IND/10 *	500mg		India	15	Gauribidnur
Met/IND/11	400mg	Yes	India	10	Gauribidnur
Met/IND/12	400mg	Yes	India	10	Gauribidnur
Met/IND/13	500mg	Yes	India	10	Gauribidnur
Met/IND/14	500mg	Yes	India	10	Gauribidnur
Met/IND/15 *	1000mg		India	10	Gauribidnur
Met/IND/16	500mg		India	20	Gauribidnur
Met/SA/1	500mg		Saudi Arabia	50	Medina

* Sustained Release Formulations

Table 4.6: Antimalarial tablet samples analysed

Name of Tablet	Dosage	API	Country of Origin	Number of Tablets
AM/UK/1	250mg	Chloroquine Phosphate	UK	20
AM/GHA/1	500mg/25mg	Sulphadoxine + Pyrimethamine	Ghana	6
AM/NEP/1	250mg	Chloroquine Phosphate	Nepal	30
AM/NIG/1	80mg/480mg	Artemether + Lumefantrine	Nigeria	6
AM/NIG/2	80mg/480mg	Artemether + Lumefantrine	Nigeria	6
AM/NIG/3	400mg	Chloroquine Sulphate	Nigeria	5
AM/ZIM/1	20mg/120mg	Artemether + Lumefantrine	Zimbabwe	12
AM/ZIM/2	20mg/480mg	Artemether + Lumefantrine	Zimbabwe	12
AM/ZIM/3	500mg/25mg	Sulphadoxine + Pyrimethamine	Zimbabwe	5
AM/ZIM/4	300mg	Quinine Sulphate	Zimbabwe	5

4.3 Sample Preparation and Analysis

This section details the preparation of calibration mixtures of API's and selected excipients in dry form for the qualitative and quantitative analysis of tablet samples. Details of the techniques needed for individual instrumentation are also detailed.

4.3.1 Calibration Standard Preparation

4.3.1.1 *ATR FTIR Spectroscopy*

Calibration standard mixtures of atenolol and microcrystalline cellulose (MCC) were prepared as shown in Table 4.7. Calibration standard mixtures of metformin hydrochloride and maize starch were prepared as shown in Table 4.8. The calibration standards were mixed and ground for 2 minutes using an agate pestle and mortar. The differences in calibration standards for the two API's are due to the amount of API in the tablet; atenolol tablets typically contain ~25% API w/w and metformin hydrochloride 70-90% API w/w.

Table 4.7: Calibration standard mixture concentrations of atenolol for ATR FTIR analysis

Theoretical Atenolol Content (%)	Actual Atenolol Content (%)	Weight of Atenolol (mg)	Weight of MCC (mg)
0	0	0	100
5	4.9	4.9	94.5
10	9.4	9.5	91.2
20	20.4	20.8	81.3
25	24.7	24.9	76.1
30	30.2	30.7	70.9
40	41.1	42.0	60.3
50	49.9	50.8	51.1
60	60.4	60.2	39.5
75	73.6	75.5	27.1
100	100	100	0

Table 4.8: Calibration standard mixture concentrations of metformin hydrochloride for ATR FTIR analysis

Theoretical Metformin Hydrochloride Content (%)	Actual Metformin Hydrochloride Content (%)	Weight of Metformin Hydrochloride (mg)	Weight of Maize Starch (mg)
0	0	0	100
10	7.7	7.3	87.7
20	18.4	18.4	81.4
30	31.8	33.2	71.1
40	39.4	59.9	39.1
50	49.8	50.3	49.9
60	59.2	59.7	41.2
70	69.4	70.2	31.0
80	78.8	79.7	21.5
90	88.5	89.8	11.7
100	100	100	0

4.3.1.2 *Raman Spectroscopy*

For the purpose of Raman spectroscopy, a different set of calibration standard mixtures were analysed, this was due to the effect of fluorescence with the excipient MCC. Calibration standard mixtures of atenolol and lactose were prepared as shown in Table 4.9. Calibration standard mixtures of paracetamol and maize starch were prepared as shown in Table 4.10. The calibration standards were mixed and ground for 2 minutes using an agate pestle and mortar.

Table 4.9: Calibration standard mixture concentrations of atenolol for Raman spectroscopy

Theoretical Atenolol Content (%)	Actual Atenolol Content (%)	Weight of Atenolol (mg)	Weight of Lactose (mg)
0	0	0	100
10	9.9	9.7	88.3
20	19.2	18.8	79.8
30	30.5	31.2	71.0
40	40.7	41.0	60.2
50	49.3	49.3	50.7
60	59.8	59.7	40.1
70	70.4	70.2	29.5
80	78.8	80.8	21.7
90	90.7	85.4	8.8
100	100	100	0

Table 4.10: Calibration standard mixture concentrations of paracetamol for Raman spectroscopy

Theoretical Paracetamol Content (%)	Actual Paracetamol Content (%)	Weight of Paracetamol (mg)	Weight of Maize Starch (mg)
0	0	0	100
10	11.1	10.9	87.6
20	20.3	20.5	80.6
30	30.4	30.5	69.9
40	39.4	40.8	62.7
50	50.9	52.1	50.3
60	61.3	63.2	39.9
70	70.1	70.1	29.9
80	79.4	80.1	20.8
90	86.8	90.4	13.8
100	100	100	0

4.3.2 UV Sample Preparation and Analysis

4.3.2.1 *Atenolol*

The sample preparation for the UV determination of atenolol in tablet dosage forms was adapted and modified from the British Pharmacopoeia 2017 assay method for atenolol tablets (British Pharmacopeia 2017b). A single tablet was crushed using an agate pestle and mortar and the powder transferred to a 50ml volumetric flask using 30ml of HPLC grade methanol. The resulting suspension was heated to 60ºC and shaken for 15 minutes in a shaking water bath. The solutions were then cooled and diluted to volume (50ml) with HPLC grade methanol; this was then filtered using Whatman GF/C glass microfibre filter paper. A suitable volume of filtrate was then diluted with methanol to produce a solution containing 0.01% w/v of atenolol. The absorbance of the resulting solution was measured.

4.3.2.2 *Metformin Hydrochloride*

The sample preparation for the UV determination of metformin hydrochloride in tablet dosage forms was adapted and modified from the British Pharmacopoeia 2017 assay method for metformin hydrochloride tablets (British Pharmacopeia 2017c). Samples were weighed and crushed using an agate pestle and mortar. A quantity of powder containing 0.1g of metformin hydrochloride was shaken with 70ml of HPLC grade water for 15 minutes. The solutions were then diluted to volume (100ml) with HPLC grade water; this was then filtered using Whatman Grade 1 cellulose filter paper. The first 20mls of filtrate was discarded; 10ml of the subsequent filtrate was then diluted to 100ml with HPLC grade water. A further dilution of the resulting solution (10ml) to 100ml with HPLC grade water was performed. The absorbance of the resulting solution was measured.

4.3.3 ATR FTIR Specimen Preparation and Analysis

Both the tablet and excipient samples for ATR FTIR Spectroscopy were crushed and ground into a fine powder for a minimum of two minutes using an agate pestle and mortar and directly analysed using ATR FTIR.

The reference and tablet samples were analysed using the ATR accessory with a background correction. Samples were analysed in absorbance mode, measurements were taken on a small amount of sample deposited on the crystal. Each sample was analysed 5 times, with identical pressure applied for all measurements. Each spectrum consists of 30 scans. After each sample

was analysed the crystal was cleaned with isopropanol and allowed to dry in ambient air. A background of 30 scans was performed after each sample group had been analysed.

Each spectrum was baseline corrected before processing for quantification.

4.3.3.1 *Reproducibility of Data*

To ensure the reproducibility of the data, five replicates of each sample were analysed. Figure 4.1 shows the reproducibility for an analytical standard (atenolol). The spectra are in good agreement with one another. To prevent repetition in the thesis, spectra shown in Chapter 5 and 6 will only show a single spectrum for each sample rather than the five replicates.

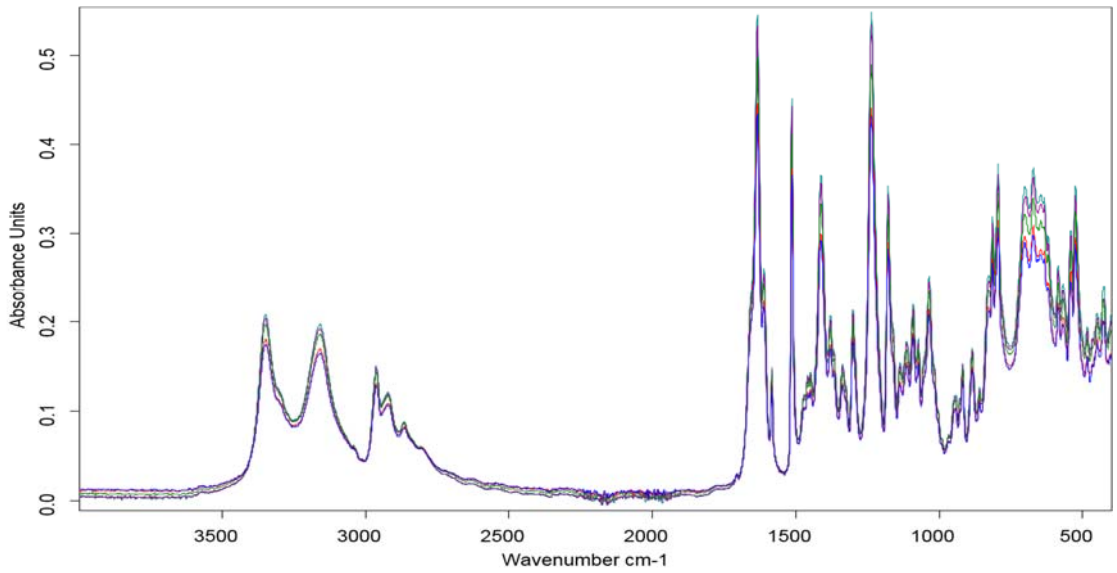

Figure 4.1: ATR FTIR Spectra of atenolol analytical reference, 1 (Blue), 2 (Red), 3 (Green), 4 (Purple) and 5 (Cyan)

4.3.3.2 *Analysis of calibration mixtures*

In order to generate a calibration plot for the calibration standard data, four peak areas characteristic to atenolol were integrated using the OPUS software. These were: Area 1) 3009 – 2893cm^{-1}, 2) 1530 – 1490cm^{-1}, 3) 1272 – 1193cm^{-1} and 4) 858 – 758cm^{-1} (Figure 4.2).

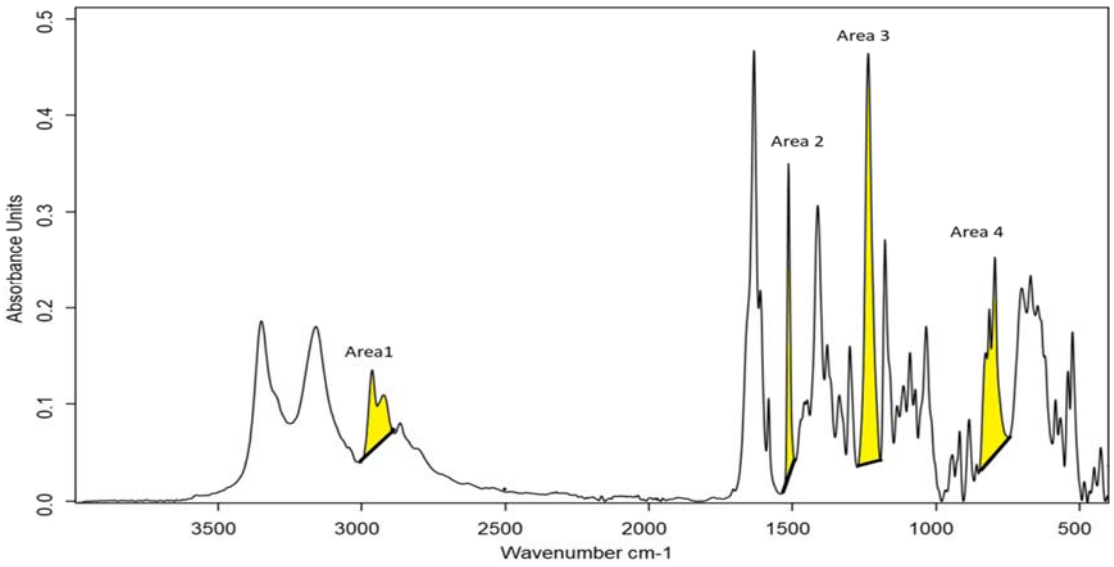

Figure 4.2: ATR FTIR spectrum of atenolol with integration areas highlighted

The areas used for metformin hydrochloride were: Area 1) 1678 – 1605cm^{-1}, 2) 1605 – 1501cm^{-1}, 3) 1501 – 1432cm^{-1} and 4) 1432 – 1341cm^{-1} (Figure 4.3).

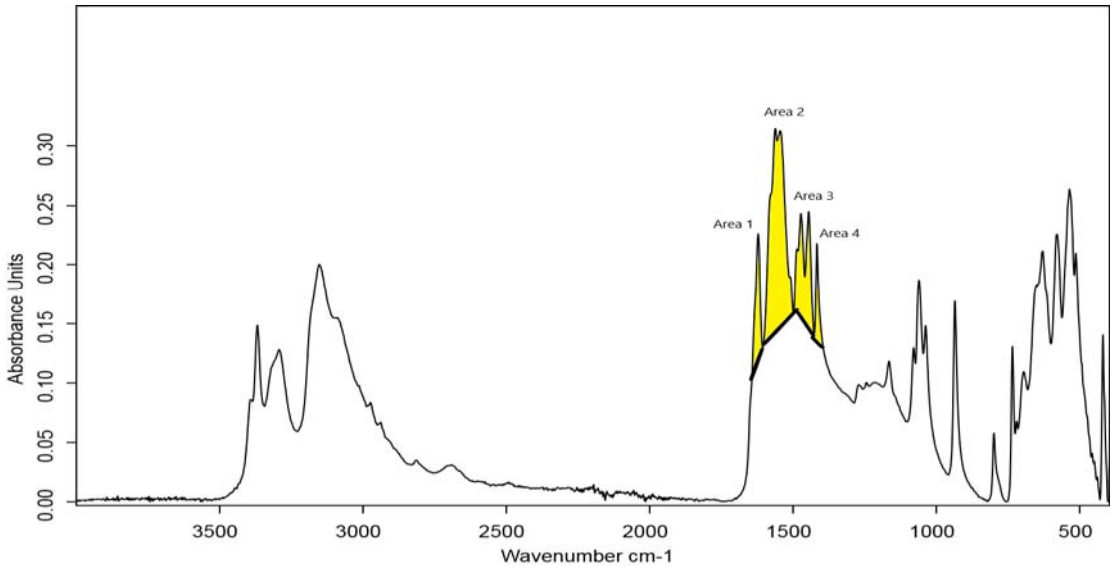

Figure 4.3: ATR FTIR spectrum of metformin hydrochloride with integration areas highlighted

The peak areas were integrated using three different methods (Figure 4.4):

- Method A – The area between the band, abscissa and the frequency limits defined is integrated,
- Method B – The area above the line drawn between the peaks of the two defined frequency limits is integrated,
- Method K – The peak intensity relative to the local baseline.

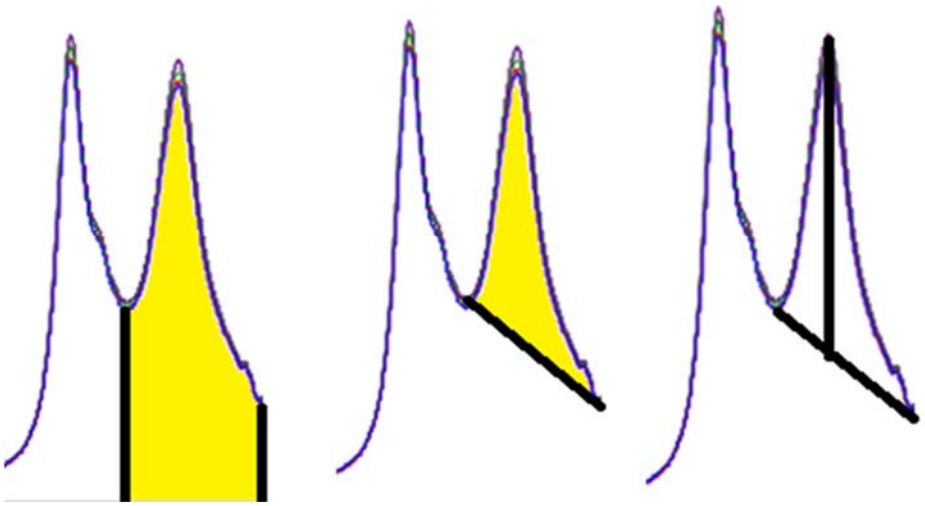

Figure 4.4: Different Integration Methods, a) Method A, b) Method B and c) Method K

Calibration plots were plotted in Microsoft Excel using the mean integrated peak areas, for all four areas and integration methods.

4.3.4 Raman Sample Preparation and Analysis

Samples for Raman spectroscopy can be either the whole tablet or a finely ground powder. For the purpose of this study, both types of sample preparation were analysed. For tablets that were either film coated or had a coloured coating, the effects of the tablet coating on the spectra were studied by comparing a surface spectrum with that from a crushed sample. To obtain the latter the coating was removed and the remaining tablet crushed and ground into a fine powder using an agate pestle and mortar and directly analysed. For some samples the coating was also crushed into the main body of the sample and the total powder analysed.

4.3.4.1 *Handheld Raman Spectrometers*

Two different handheld spectrometers were used for this research; these were a Bruker BRAVO and Metrohm MIRA-3 spectrometers.

A BRAVO Raman spectrometer was used to analyse the atenolol samples. The samples were analysed in absorbance mode, measurements were taken on either a whole or a crushed tablet deposited over the bag tip.

A MIRA-3 Raman spectrometer was used to analyse the atenolol and metformin hydrochloride samples. Measurements were taken on a crushed tablet placed in a glass curvette using the curvette holder tip.

4.3.4.2 *Benchtop Raman Spectrometer*

A FORAM 2 Raman spectrometer was used to analyse the atenolol, metformin hydrochloride and antimalarial samples. Measurements were taken on whole tablets placed under the objective lens and focused using the XYZ translation stage. The results were compared with powdered samples analysed by the FORAM-2.

4.3.4.3 *Reproducibility of Data*

To ensure the reproducibility of the data, five replicates of each sample were analysed. To prevent repetition in the thesis, spectra shown in Chapter 5 will only show a single spectrum for each sample rather than the five replicates.

4.3.5 DIP Sample Preparation and Analysis

The tablet samples analysed by DIP needed to be a fine powder. Samples were crushed into a fine powder using an agate pestle and mortar. A small amount of sample was then transferred to a flared vial (Bruker, Coventry, UK) using a Platinum wire adhered to a glass capillary tube.

An empty flared sample vial was inserted into the heated tip of the probe and inserted into the MS through the vacuum interlock and ran as a blank three times. The sample was then transferred to the blank flared sample vial using a platinum wire adhered to a glass capillary tube and analysed. The atenolol samples were heated over a temperature range of 30ºC to 250ºC, with a 30ºC a minute ramp. The source temperature was set at 300ºC; with the ionising electron energy set at 70eV and the run time was set to 10 minutes. The antimalarial samples were heated over a temperature range of 40ºC to 350ºC, with a 30ºC a minute ramp. The source temperature was set at 250ºC; with the ionising electron energy set at 70eV and the run time was set to 10.3 minutes. These were collated to produce the thermal ionic fingerprint of the analytes.

4.3.5.1 *Reproducibility of Data*

To ensure the reproducibility of the data, three replicates of each sample were analysed. To prevent repetition in the thesis, spectra shown in Chapter 5 will only show a single spectrum for each sample rather than the three replicates.

4.3.6 ASAP Sample Preparation and Analysis

The tablet samples analysed by ASAP needed to be a fine powder. Samples were crushed into a fine powder using an agate pestle and mortar. A small amount of sample was then transferred to the closed end of a melting point tube. Excess powder was removed before the probe was inserted directly into the ionisation region and the flow of heated nitrogen (300ºC) volatises the API/'s. Ionisation occurs by protonation in the cornea discharge field. Ions are focussed into the Advion Compact MS for analysis.

4.3.7 SEM/EDX Specimen Preparation and Analysis

Materials selected for SEM/ EDX must be compatible (non-volatile) with the vacuum in the SEM specimen chamber and stable when irradiated with a beam of high energy electrons (Nichols, 2012).

Cross-sections of the tablets were prepared by cutting the tablets with a tablet cutter (Safe and Sound Health, Hampshire, UK). The cross-sections were then mounted onto 12.5 mm aluminium stubs (Agar Scientific, Stansted, UK), using Leit-C carbon cement (Agar Scientific, Stansted, UK). The samples were then sputtered coated with 15nm of gold using a Q150RS sputter coater (Quorum Technologies, Sussex, UK); this was to make the samples conductive.

The samples were analysed under high vacuum, as the samples were gold coated and therefore conductive. The samples were analysed with a beam accelerating voltage of 20kV, 20µm beam aperture, a probe current of 100pA and a working distance of 8.5mm. It has been observed by some research groups and from experience that images for samples analysed under high vacuum are of a better quality and higher resolution than of those analysed under variable pressure (Nichols, 2012 and Stokes 2008). Images were recorded using both a SE and BSE detectors, SE images are more three-dimensional and give topographical information, whilst BSE images can appear flatter due to the mounting of the detector, but will give compositional information.

For EDX the same conditions were used, with the exception of the probe current, which was set at 1000pA; this was to facilitate the optimum collection of X-rays. When analysing non-conductive samples by EDX, it is known that carbon coating is better than gold, this is due to the gold peaks interfering with some elements. For the purpose of this research, it was decided that as most tablets are organic the carbon peaks would be of interest and that the gold peaks did not interfere with any of the elements being analysed. Elemental data was acquired using INCA software (Oxford Instruments, High Wycombe, UK); mapping and quantitative data were acquired over 20 scans.

5 API Identification and Discussion

This chapter is devoted to the identifying the API's based on results obtained from the experimental work detailed in the previous chapter. Results are presented according to identification of an API in a whole tablet and a crushed tablet sample. Within these two sections, results are grouped together by technique.

It has been stated by previous studies, in particular by Bugay and Brush (2010), that in the development of a chemical identification assay, obtaining a pure reference spectrum under the same conditions is the most important step (Bugay and Brush, 2010). Without an authentic reference spectrum, the validity of the chemical identification assay is susceptible (Bugay and Brush, 2010). For applications such as the study of substandard and falsified medicines 'in the field', the appropriate reference material is in fact the formulated tablet. Furthermore, these reference tablets can be either the innovator sample or acceptable generic formulations.

5.1 Spectroscopic

5.1.1 Reference Spectra of API and Excipients

Reference spectra was obtained for the most common tablet excipients as discussed in Chapter 2 and for analytical grade atenolol, metformin hydrochloride, chloroquine phosphate and quinine sulphate references using ATR FTIR and Raman spectroscopy. The reference material for both API and excipients was crushed and ground into a fine powder.

5.1.1.1 *ATR FTIR Spectroscopy*

Figure 5.1 shows the ATR FTIR spectra for common excipients in atenolol tablets, Figure 5.2 shows the ATR FTIR spectra for common excipients in metformin hydrochloride tablets and Figure 5.3 shows the ATR FTIR spectra for common excipients in antimalarial tablets.

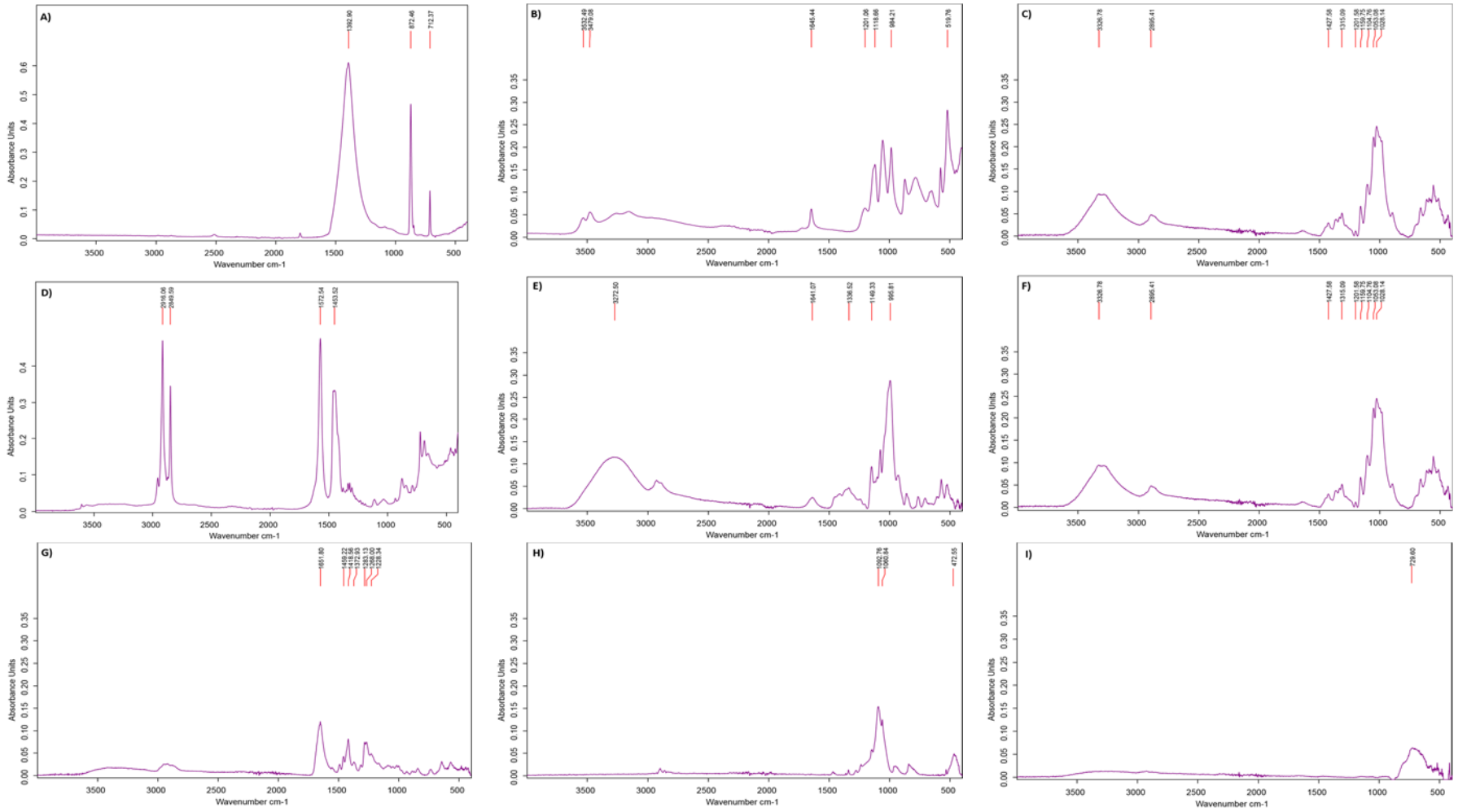

Figure 5.1: ATR FTIR spectra for common excipients in atenolol tablets, A) calcium carbonate, B) dicalcium phosphate, C) lactose monohydrate, D) magnesium stearate, E) maize starch, F) MCC, G) povidone, H) silicon dioxide (colloidal) and I) titanium dioxide

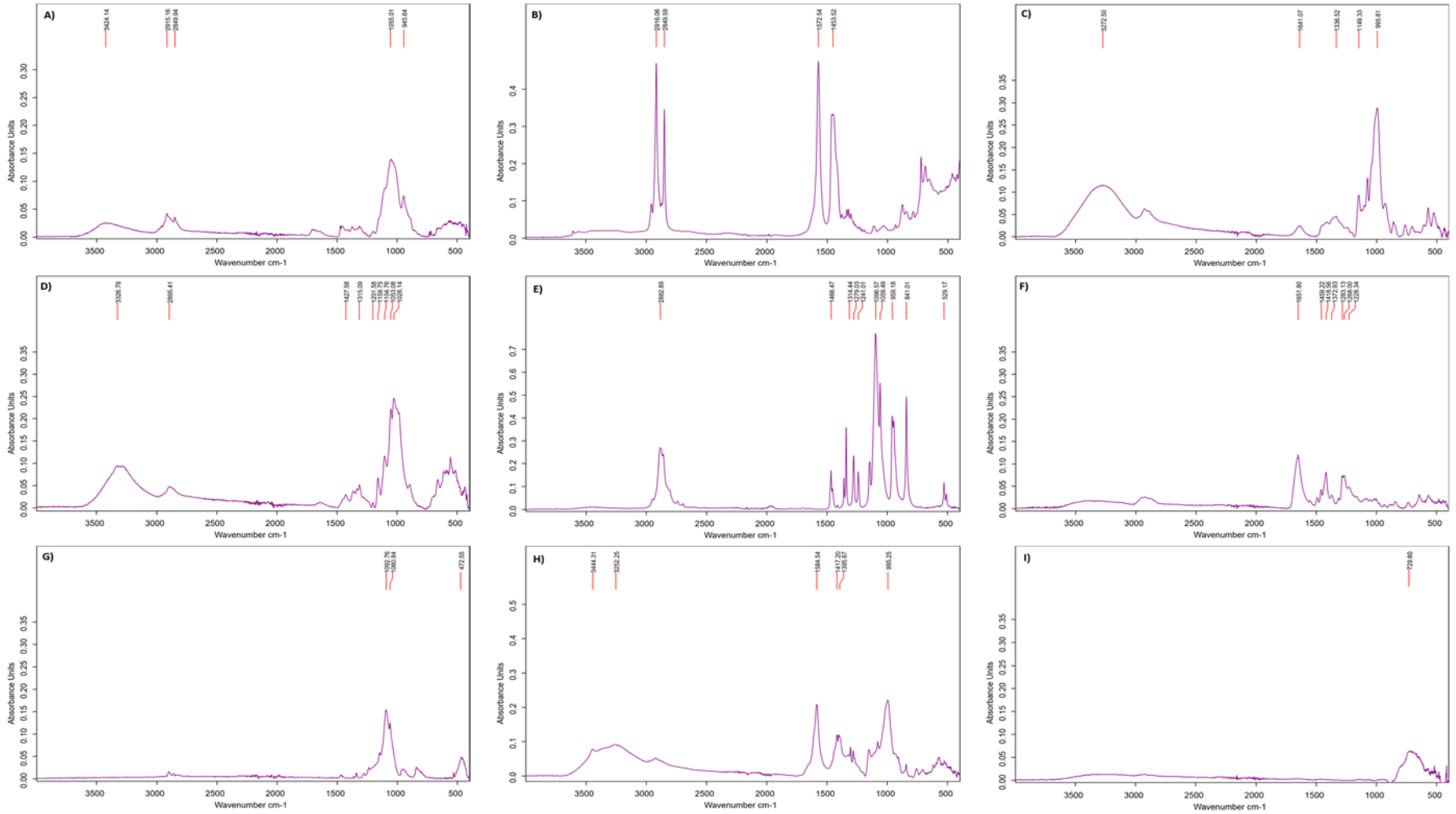

Figure 5.2: ATR FTIR spectra for common excipients in metformin hydrochloride tablets, A) hypromellose, B) magnesium stearate, C) maize starch, D) MCC, E) PEG, F) povidone, G) silicon dioxide (colloidal) H) sodium starch glycolate and I) titanium dioxide

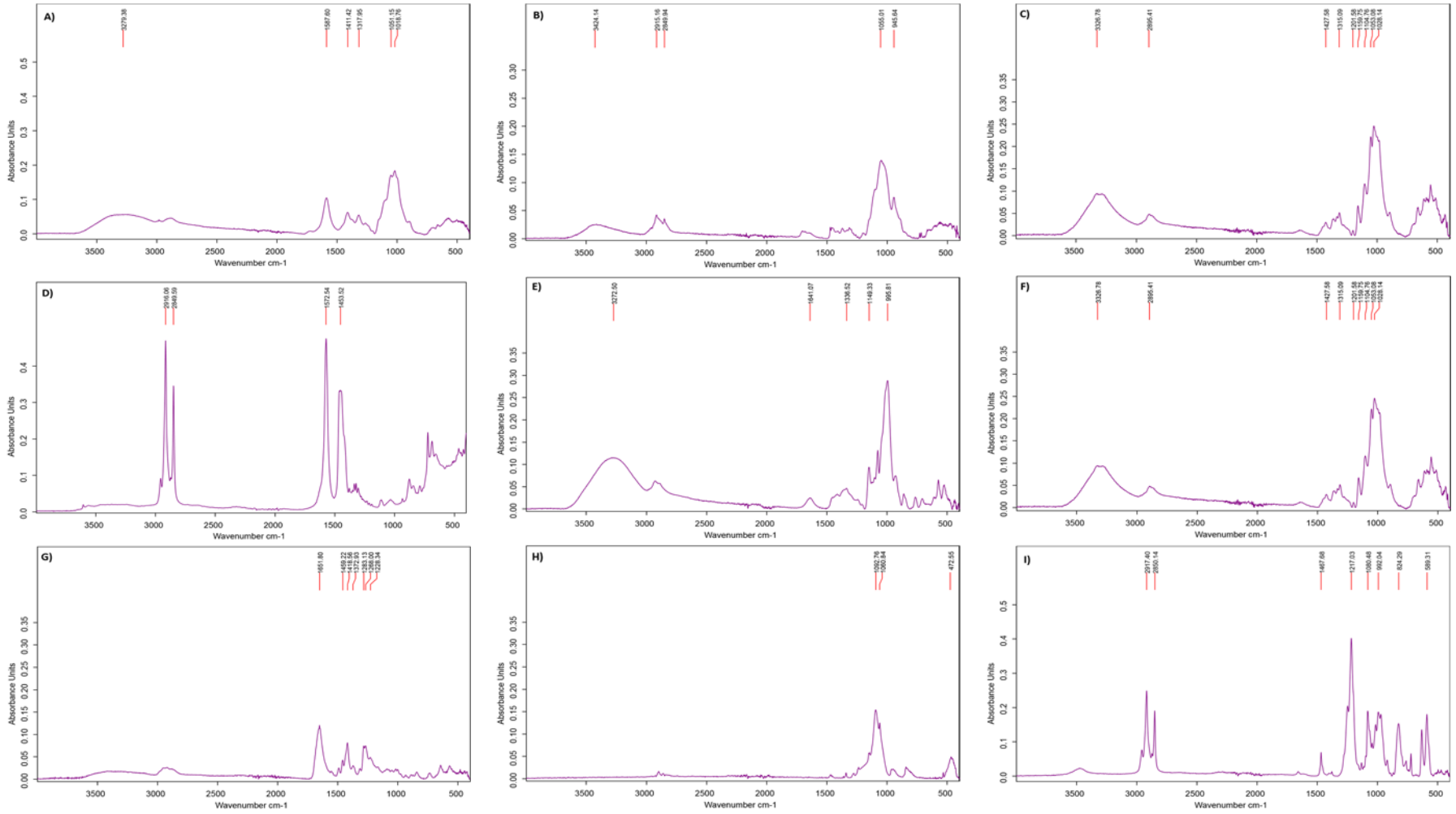

Figure 5.3: ATR FTIR spectra for common excipients in antimalarial tablets, A) croscarmellose sodium, B) hypromellose, C) lactose monohydrate, D) magnesium stearate, E) maize starch, F) MCC, G) povidone, H) silicon dioxide (colloidal) and I) SLS

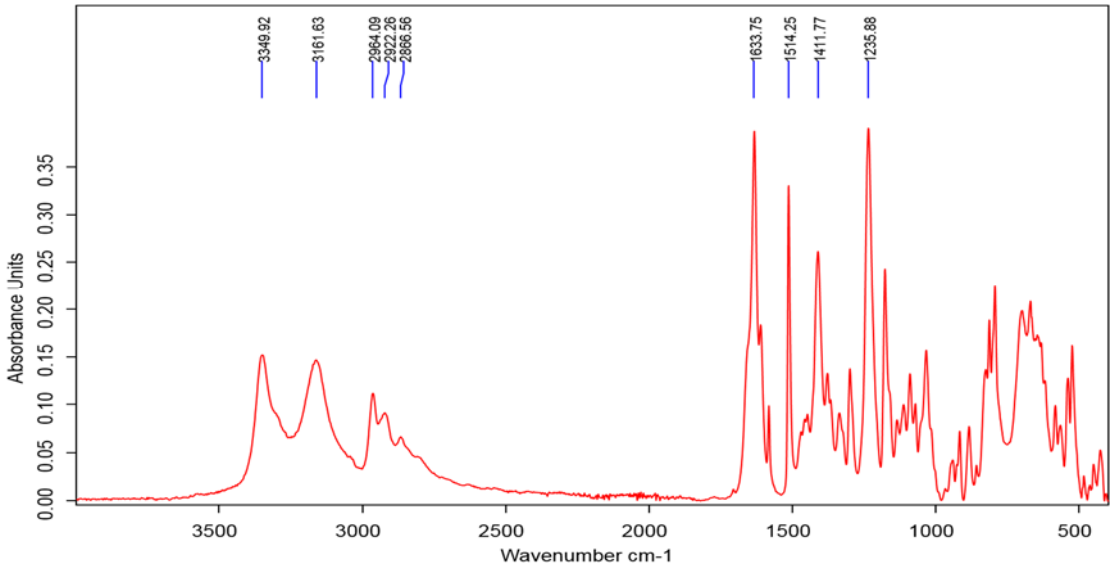

Figure 5.4: ATR FTIR spectrum of atenolol (analytical standard)

The ATR FTIR spectrum for the atenolol analytical standard (Figure 5.4) exhibited characteristic peaks for atenolol at 3159 – 3347cm^{-1} (hydroxyl and amine) (El-Leithy *et al*., 2012), 2866 – 2966cm^{-1} (CH, aliphatic) (El-Leithy *et al*., 2012), 1633cm^{-1} (C=O carbonyl) (Jalindar *et al*., 2001), 1514cm^{-1} (aromatic ring) and 1412cm^{-1} (nitro compound) (Edukondalu *et al*., 2011).

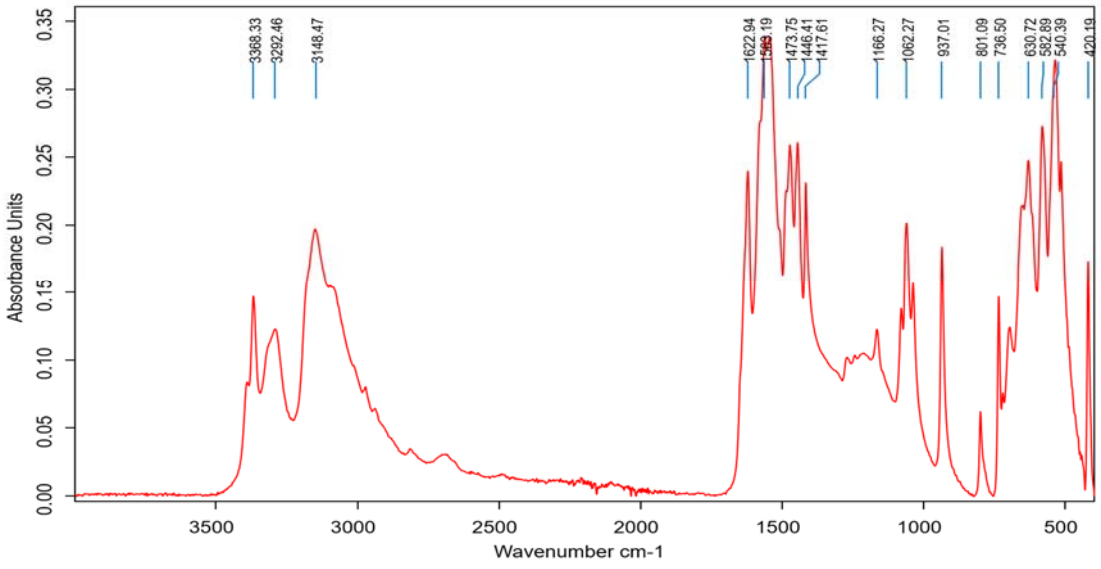

Figure 5.5: ATR FTIR spectrum of metformin hydrochloride (analytical standard)

The ATR FTIR spectrum for the metformin hydrochloride analytical reference standard (Figure 5.5) exhibited characteristic peaks for metformin hydrochloride at 3148 – 3368cm^{-1} (Primary Amine) (Roselet and Premakumari, 2015), 1417 – 1622cm^{-1} (NH) (Roselet and Premakumari, 2015 and Sheela *et al*., 2010) 1062 – 1166cm^{-1} (CN Amine) (Sheela *et al*., 2010) and 420 – 582cm^{-1} (C-N-C deformation) (Sheela *et al*., 2010).

Using the excipient spectra and comparing it to the analytical grade atenolol standard spectrum, we can summarise which excipients mask characteristic atenolol peaks. This is summarised in Table 5.1.

Table 5.1: Summary of excipients and their differentiation with characteristic atenolol peaks

	Atenolol Reference Peaks (cm^{-1})							
Excipient	**3347**	**3159**	**2966**	**2923**	**1633**	**1515**	**1412**	**1235**
Calcium carbonate	✓	✓	✓	✓	✓	✓	×	✓
Calcium phosphate	✓	✓	✓	✓	✓	✓	✓	✓
Carnauba wax	✓	✓	✓	×	✓	✓	✓	✓
Citric acid	✓	✓	✓	✓	✓	✓	✓	✓
Croscarmellose sodium	✓	✓	✓	✓	✓	✓	✓	✓
Colloidal silicon dioxide	✓	✓	✓	✓	✓	✓	✓	✓
HPMC	✓	✓	✓	✓	✓	✓	✓	✓
Iron Oxide	✓	✓	✓	✓	✓	✓	✓	✓
Lactose *	✓	✓	✓	×	✓	✓	✓	✓
Magnesium stearate	✓	✓	×	×	✓	✓	×	✓
MCC	✓	✓	✓	✓	✓	✓	✓	✓
PEG	✓	✓	✓	×	✓	✓	✓	×
Povidone	✓	✓	✓	✓	✓	✓	✓	✓
SLS	✓	✓	×	×	✓	✓	✓	×
Sodium starch glycolate	✓	✓	✓	✓	✓	✓	×	✓
Starch [#]	✓	✓	✓	✓	✓	✓	✓	✓
Stearic acid	✓	✓	×	×	✓	✓	×	×
Talc	✓	✓	✓	✓	✓	✓	✓	✓
Tartaric acid	×	✓	×	×	✓	✓	✓	✓
Titanium dioxide	✓	✓	✓	✓	✓	✓	✓	✓

✓ - Atenolol peaks can be observed fully/partially × - Atenolol peaks inhibited

* Lactose including lactose monohydrate 80M, Grannulac 200, lactose EP granular, Refined lactose and Tablettose.

[#] Starch including corn, maize and potato.

Using the excipient spectra and comparing it to the analytical grade metformin hydrochloride standard spectrum, we can summarise which excipients mask characteristic metformin hydrochloride peaks. This is summarised in Table 5.2.

Table 5.2: Summary of excipients and their differentiation with characteristic metformin hydrochloride peaks

	Metformin hydrochloride Reference Peaks (cm^{-1})								
Excipient	**3368**	**3148**	**1623**	**1418**	**1166**	**1063**	**937**	**583**	**420**
Calcium carbonate	✓	✓	✓	×	✓	✓	✓	✓	✓
Calcium phosphate	×	×	×	✓	✓	×	✓	×	×
Carnauba wax	✓	✓	✓	✓	×	✓	✓	✓	✓
Citric acid	×	×	×	×	×	×	×	×	✓
Croscarmellose sodium	×	×	×	×	✓	×	✓	✓	✓
Colloidal silicon dioxide	✓	✓	✓	✓	✓	×	✓	✓	✓
HPMC	✓	✓	✓	✓	×	×	✓	✓	✓
Iron Oxide	✓	✓	✓	✓	✓	✓	✓	×	×
Lactose *	×	×	✓	✓	✓	×	✓	✓	✓
Magnesium stearate	✓	✓	✓	×	✓	✓	✓	✓	✓
MCC	✓	✓	✓	✓	×	✓	×	✓	✓
PEG	✓	✓	✓	✓	×	×	×	✓	✓
Povidone	✓	✓	✓	✓	✓	✓	✓	✓	✓
SLS	✓	✓	✓	✓	✓	×	✓	×	✓
Sodium starch glycolate	✓	✓	✓	✓	✓	✓	✓	✓	✓
Starch [#]	×	×	✓	✓	×	×	×	×	✓
Stearic acid	✓	✓	✓	✓	✓	✓	×	✓	✓
Talc	✓	✓	✓	✓	✓	×	×	✓	×
Tartaric acid	✓	✓	✓	✓	×	×	×	×	✓
Titanium dioxide	✓	✓	✓	✓	✓	✓	✓	×	×

✓ - Metformin hydrochloride peaks can be observed fully/partially

× - Metformin hydrochloride peaks inhibited

* Lactose including lactose monohydrate 80M, Grannulac 200, lactose EP granular, Refined lactose and Tablettose.

[#] Starch including corn, maize and potato.

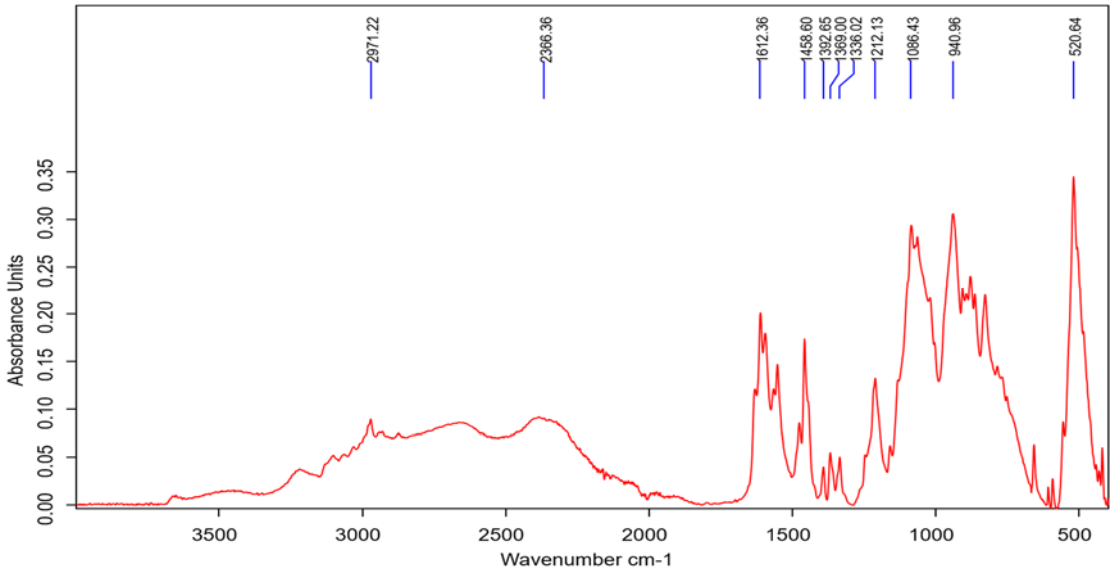

Figure 5.6: ATR FTIR spectrum of chloroquine phosphate (analytical standard)

The ATR FTIR spectrum for the chloroquine phosphate analytical standard (Figure 5.6) exhibited characteristic peaks for chloroquine phosphate at 2971cm^{-1}, 1612cm^{-1}, 1459cm^{-1} and 1212cm^{-1} (Kozicki *et al.*, 2015).

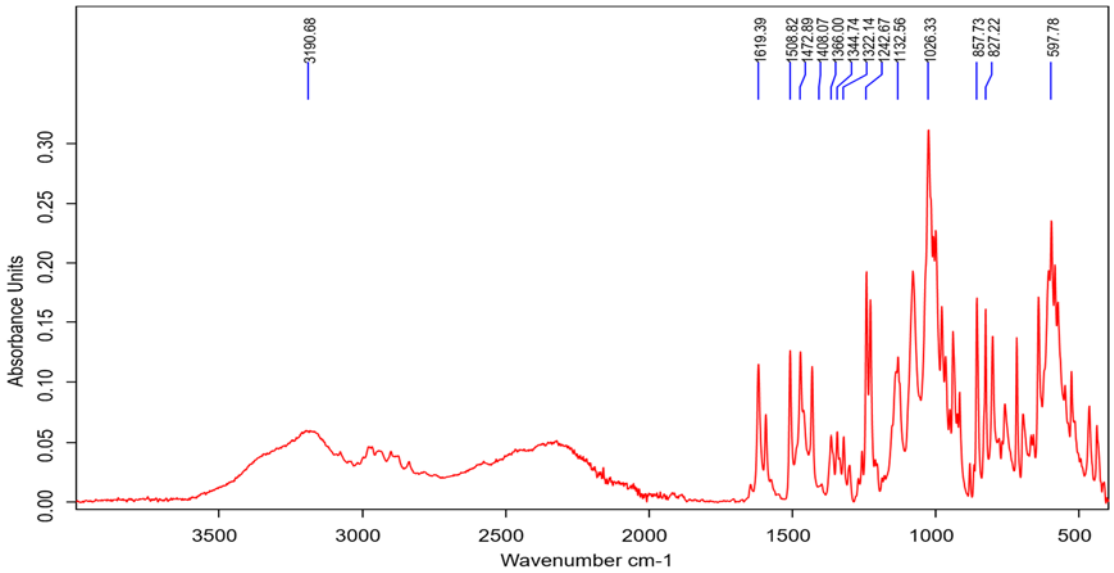

Figure 5.7: ATR FTIR spectrum of quinine sulphate (analytical standard)

The ATR FTIR spectrum for the quinine sulphate analytical standard (Figure 5.7) exhibited characteristic peaks for quinine sulphate at 3191cm^{-1}, 1619cm^{-1}, 1509cm^{-1}, 1243cm^{-1} and 1026cm^{-1} (Karan *et al.*, 2012).

Using the excipient spectra and comparing it to the analytical grade chloroquine phosphate standard spectrum, we can summarise which excipients mask characteristic chloroquine phosphate peaks. This is summarised in Table 5.3.

Table 5.3: Summary of excipients and their differentiation with characteristic chloroquine phosphate peaks

	Chloroquine Phosphate Reference Peaks (cm^{-1})			
Excipient	**2971**	**1612**	**1459**	**1212**
Calcium carbonate	✓	✓	×	×
Calcium phosphate	✓	✓	✓	✓
Carnauba wax	✓	✓	×	×
Citric acid	×	✓	✓	×
Croscarmellose sodium	×	✓	✓	✓
Colloidal silicon dioxide	✓	✓	✓	✓
HPMC	✓	✓	✓	✓
Iron Oxide	✓	✓	✓	✓
Lactose *	×	✓	✓	✓
Magnesium stearate	×	×	×	✓
MCC	✓	✓	✓	✓
PEG	✓	✓	×	✓
Povidone	✓	✓	✓	✓
SLS	×	✓	×	×
Sodium starch glycolate	✓	×	✓	✓
Starch [#]	×	✓	✓	✓
Stearic acid	×	✓	×	×
Talc	✓	✓	✓	✓
Tartaric acid	✓	✓	✓	×
Titanium dioxide	✓	✓	✓	✓

✓ - Chloroquine phosphate peaks can be observed fully/ partially

× - Chloroquine phosphate peaks masked

* Lactose including lactose monohydrate 80M, Grannulac 200, lactose EP granular, Refined lactose and Tablettose.

[#] Starch including corn, maize and potato.

Using the excipient spectra and comparing it to the analytical grade quinine sulphate standard spectrum, we can summarise which excipients mask characteristic quinine sulphate peaks. This is summarised in Table 5.4.

Table 5.4: Summary of excipients and their differentiation with characteristic quinine sulphate peaks

	Quinine Sulphate Reference Peaks (cm^{-1})				
Excipient	**3191**	**1619**	**1509**	**1243**	**1026**
Calcium carbonate	✓	✓	×	✓	✓
Calcium phosphate	×	✓	✓	✓	✓
Carnauba wax	✓	✓	✓	✓	✓
Citric acid	×	✓	✓	✓	✓
Croscarmellose sodium	×	✓	✓	✓	✓
Colloidal silicon dioxide	✓	✓	✓	✓	✓
HPMC	✓	✓	✓	✓	×
Iron Oxide	✓	✓	✓	✓	✓
Lactose *	×	✓	✓	✓	×
Magnesium stearate	✓	✓	✓	✓	✓
MCC	×	✓	✓	✓	✓
PEG	✓	✓	✓	×	✓
Povidone	✓	×	✓	✓	✓
SLS	✓	✓	✓	×	✓
Sodium starch glycolate	×	×	✓	✓	✓
Starch #	✓	✓	✓	✓	×
Stearic acid	✓	✓	✓	✓	✓
Talc	✓	✓	✓	✓	×
Tartaric acid	✓	✓	✓	✓	✓
Titanium dioxide	✓	✓	✓	✓	✓

✓ - Quinine sulphate peaks can be observed fully/partially

× - Quinine sulphate peaks inhibited

* Lactose including lactose monohydrate 80M, Grannulac 200, lactose EP granular, Refined lactose and Tablettose. # Starch including corn, maize and potato.

5.1.1.2 *Raman Spectroscopy*

Raman spectra were obtained for tablet excipients and for analytical grade atenolol, metformin hydrochloride, chloroquine phosphate and quinine sulphate references. Figure 5.8 shows the Raman spectra for common excipients tablets. The reference material for both API and excipients was crushed and ground into a fine powder.

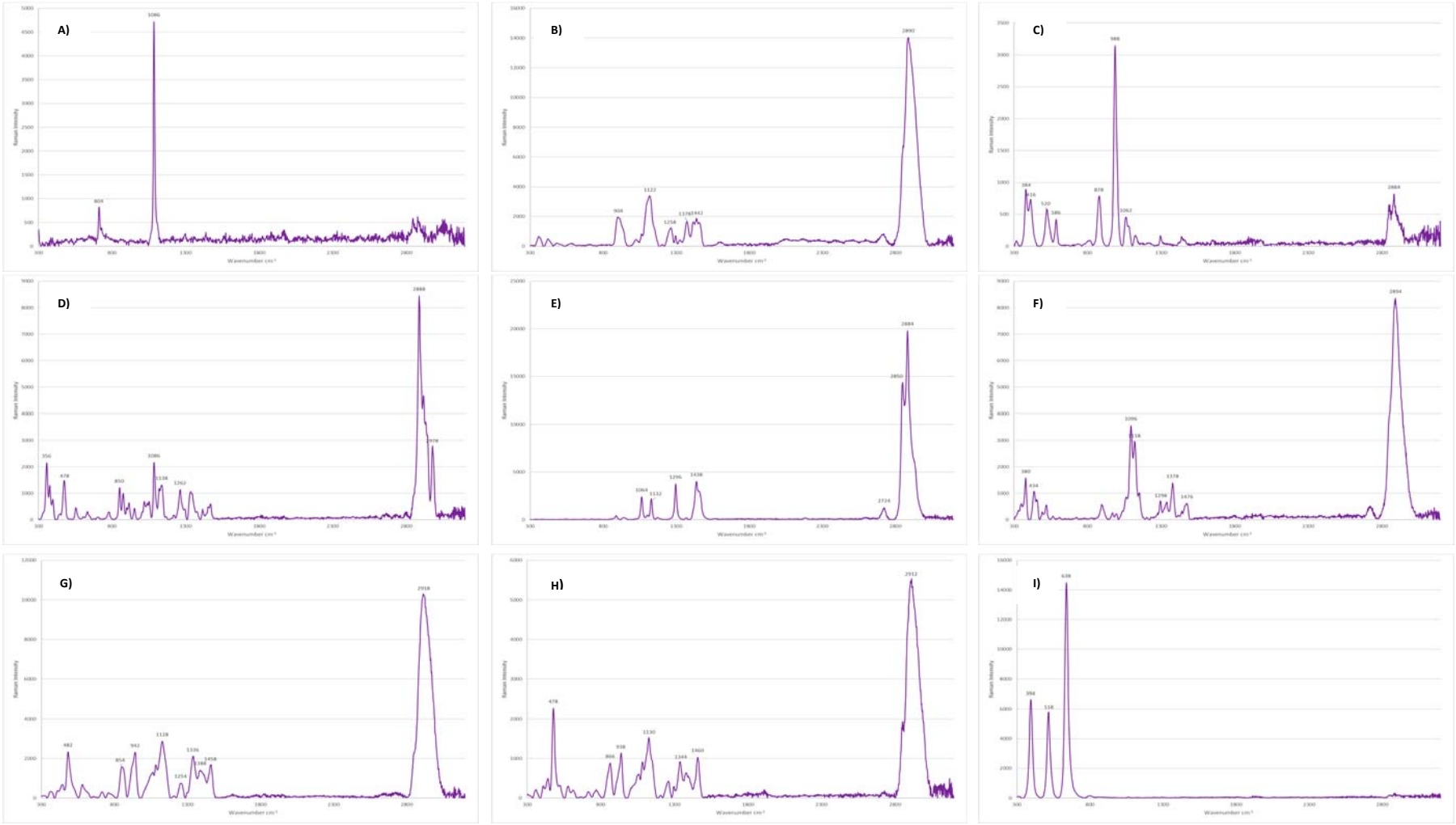

Figure 5.8: Raman spectra for common excipients, A) calcium carbonate, B) croscarmellose sodium, C) dicalcium phosphate, D) lactose monohydrate, E) magnesium stearate, F) MCC, G) sodium starch glycolate, H) starch and I) titanium dioxide

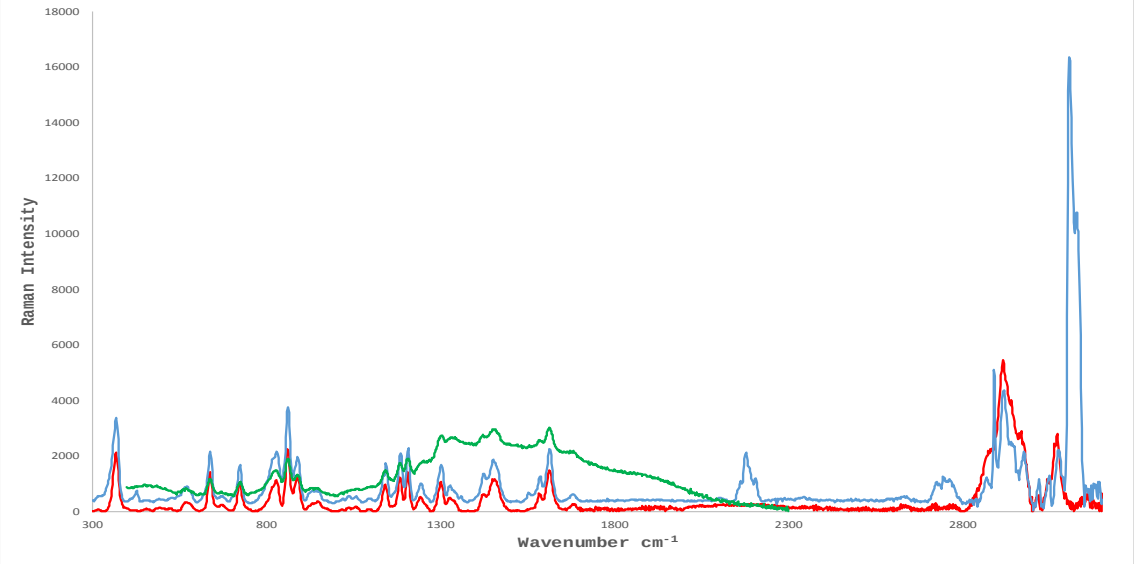

Figure 5.9: Raman spectra of atenolol (analytical standard) using BRAVO handheld spectrometer (Red), FORAM-2 spectrometer (Blue) and MIRA handheld spectrometer (Green)

The Raman spectrum for the atenolol analytical standard (Figure 5.9) exhibited characteristic peaks for atenolol at 368cm^{-1}, 637cm^{-1}, 828cm^{-1}, 1187cm^{-1}, 1209cm^{-1}, 1301cm^{-1}, 1423cm^{-1}, 1451cm^{-1}, 1615cm^{-1},1680cm^{-1} (Farças *et al*., 2016), 2916cm^{-1} and 3072cm^{-1} (Cozar *et al*., 2010). Variation within the spectra can be observed for the different spectrometers, with the FORAM-2 spectrum displaying additional peaks. Table 5.5 summarises the peaks attributed to atenolol for the different spectrometers and compares them to those in the literature.

Table 5.5: Characteristic Raman peaks of atenolol and their assignments

Peak Position (cm^{-1})						Assignment
Experimental			Literature Values			
BRAVO	**FORAM**	**MIRA-3**	**Ruperez and Laserna (1996)**	**Cozar *et al* (2010)**	**Farcaş *et al* (2016)**	
368	368			366	368	NH rocking
638	638	638		636	637	In plane ring deformation
723	722	725			732	
828	828	829	828	825	828	Out of plane bending (CH) + ring
861	863	861			859	Ring breathing
1142	1144	1141		1140	1143	In plane bending chain + ring
1185	1188	1185	1198		1183	Chain stretching
1206	1209	1207		1205	1205	Chain stretching
	1245			1244	1243	In plane bending chain
1302	1302	1301	1295	1301	1301	In plane bending CH2
	1424	1422		1424	1423	In plane bending chain
1456	1451	1453	1444	1454	1448	In plane bending CH3
1585	1586	1586	1585		1584	Ring stretching
1614	1616	1613	1609	1614	1612	Ring stretching
2916	2922			2899		Symmetric stretching CH2
3072	3108			3045		Asymmetric stretching CH3

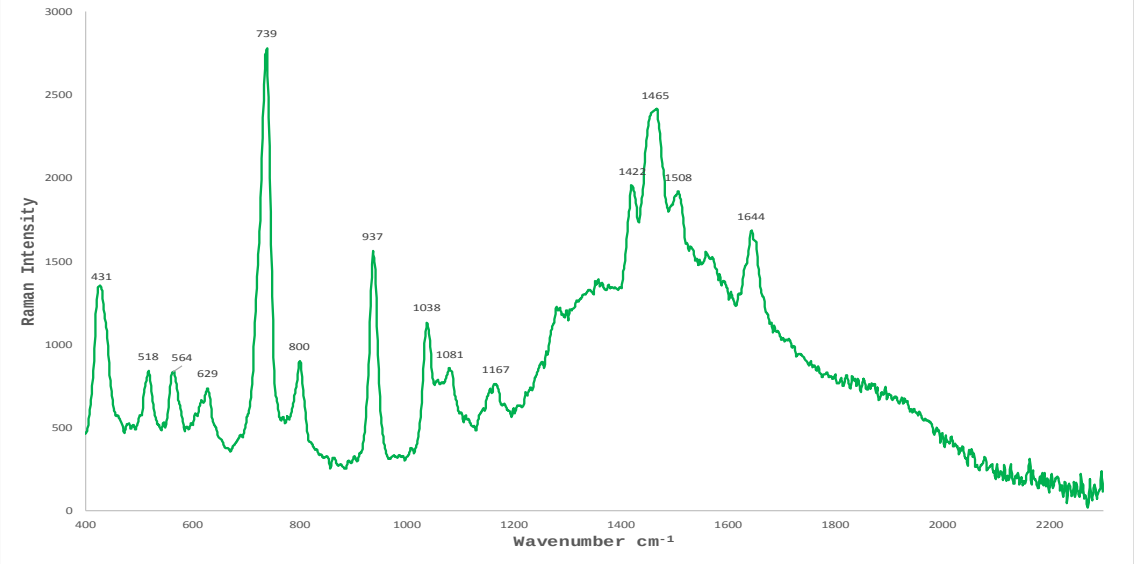

Figure 5.10: Raman spectrum of metformin hydrochloride (analytical standard) using MIRA-3 handheld spectrometer

The Raman spectra for the metformin hydrochloride reference standard (Figure 5.10) exhibits peaks that are characteristic for metformin hydrochloride at 426cm^{-1}, 518cm^{-1} and 565cm^{-1}, attributable to C-N-C deformation (Renganayaki and Srinvasan, 2011). Vibrations occurring at 628cm^{-1}, 737cm^{-1}, 800cm^{-1} and 937cm^{-1} are assigned respectively as NH_2 rocking and N-H out of plane bending and NH_2 vibration modes (Sheela *et al*, 2010, Renganayaki and Srinvasan, 2011). Peaks at 1039cm^{-1}, 1081cm^{-1}, 1168cm^{-1} are attributable to C-N stretching, whereas the peak at 1645cm^{-1} is attributable to C=N stretching (Renganayaki and Srinvasan, 2011). Vibrations occurring at 1420cm^{-1} and 1645cm^{-1} can be assigned to CH_3 asymmetric deformation (Renganayaki and Srinvasan, 2011).

Table 5.6 summarises the peaks attributed to metformin hydrochloride for the different spectrometers and compares them to those in the literature.

Table 5.6: Characteristic Raman peaks of metformin hydrochloride and their assignment

Peak Position (cm^{-1})				Assignment
Experimental		Literature Values		
FORAM	MIRA-3	Sheela *et al* (2010)	Renganayaki and Srinvasan (2011)	
	426	432	432	C-N-C deformation
518	518	524		C-N-C deformation
562	565	569	569	C-N-C deformation
	628	634		NH_2 rocking
740	737	744	744	N-H out of plane bending
796	800	806	806	N-H wagging
	937	943	943	N-H wagging
1036	1039	1043	1043	C-N stretching
	1081	1087	1087	C-N stretching
	1168	1169	1169	C-N stretching
1426	1420		1427	CH_3 asymmetric deformation
1456	1465	1472	1472	CH_3 asymmetric deformation
		1571	1571	C=N stretching
1646	1645	1653	1653	C=N stretching

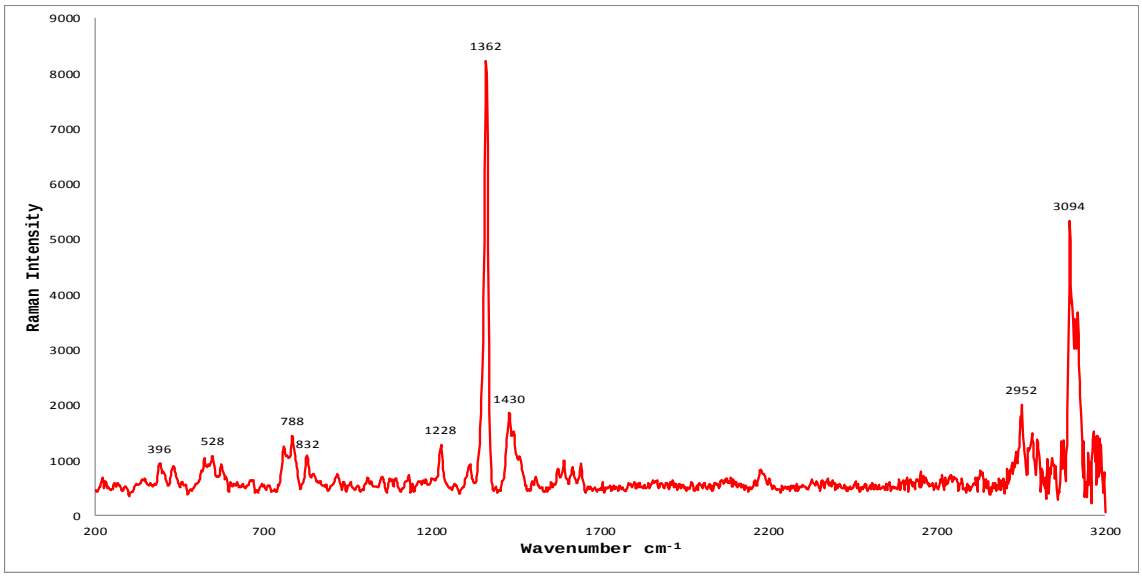

Figure 5.11: Raman spectrum of chloroquine phosphate (analytical standard) using FORAM-2 benchtop spectrometer

The Raman spectra for the chloroquine phosphate reference standard (Figure 5.11) exhibits peaks that are characteristic for chloroquine phosphate at 396cm^{-1}, attributable to C-N-C deformation. Vibrations occurring at 788cm^{-1} and 830cm^{-1} are assigned respectively as NH_2 rocking and N-H out of plane bending (Cîntă-Pînzaru *et al*., 2006). Peaks at 1362cm^{-1} and 1430cm^{-1} are attributable to C-C stretching and CH_3 asymmetric deformation (Cîntă-Pînzaru *et al*., 2006). Vibrations occurring at 2952cm^{-1} and 3094cm^{-1} can be assigned to CH_3 asymmetric stretching and N-H symmetric stretching (Cîntă-Pînzaru *et al*., 2006).

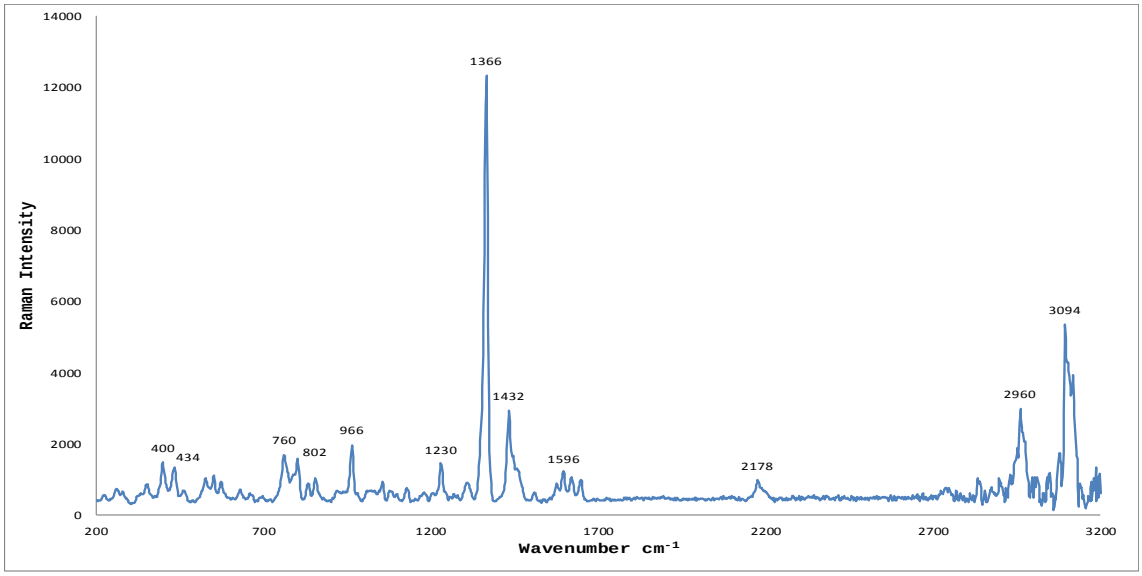

Figure 5.12: Raman spectrum of quinine sulphate (analytical standard) using FORAM-2 benchtop spectrometer

The Raman spectra for the quinine sulphate reference standard (Figure 5.12) exhibits peaks that are characteristic for quinine sulphate. The peak at 434cm^{-1} attributable to C-N-C, vibrations occurring at 760cm^{-1}, 804cm^{-1} and 966cm^{-1} are assigned respectively as NH_2 rocking and N-H out of plane bending and NH_2 vibration modes. Peaks at 1366cm^{-1} and 1432cm^{-1} are attributable to C-C stretching and CH_3 asymmetric deformation. Vibrations occurring at 2960cm^{-1} and 3094cm^{-1} can be assigned to CH_3 asymmetric stretching and N-H symmetric stretching.

5.1.1.3 *Discussion*

Using the spectra obtained for reference APIs and excipients libraries can be created in order to determine the content of a tablet. From looking at the ATR FTIR and Raman spectra, there are distinct peaks for both API and excipient and these can be used as markers for the individual component.

From analysing the ATR FTIR spectra, distinct peaks for atenolol and metformin hydrochloride with little or no interference from excipients can be observed. These peaks can potentially be used to quantify the amount of API in a tablet; this will be discussed further in the next chapter.

5.1.2 Whole Tablet Studies

The most common oral dosage form is a tablet; because of this, it is important to be able to identify an API within the physical dosage form. This capability will speed up the analysis, which would be invaluable in assessing tablet consignments.

5.1.2.1 *ATR FTIR Spectroscopy*

Whole atenolol tablets were analysed in order to see if ATR FTIR analysis produced meaningful data bearing in mind the different tablet shapes and the need for good contact with the ATR sampling region.

5.1.2.1.1 Atenolol Tablets

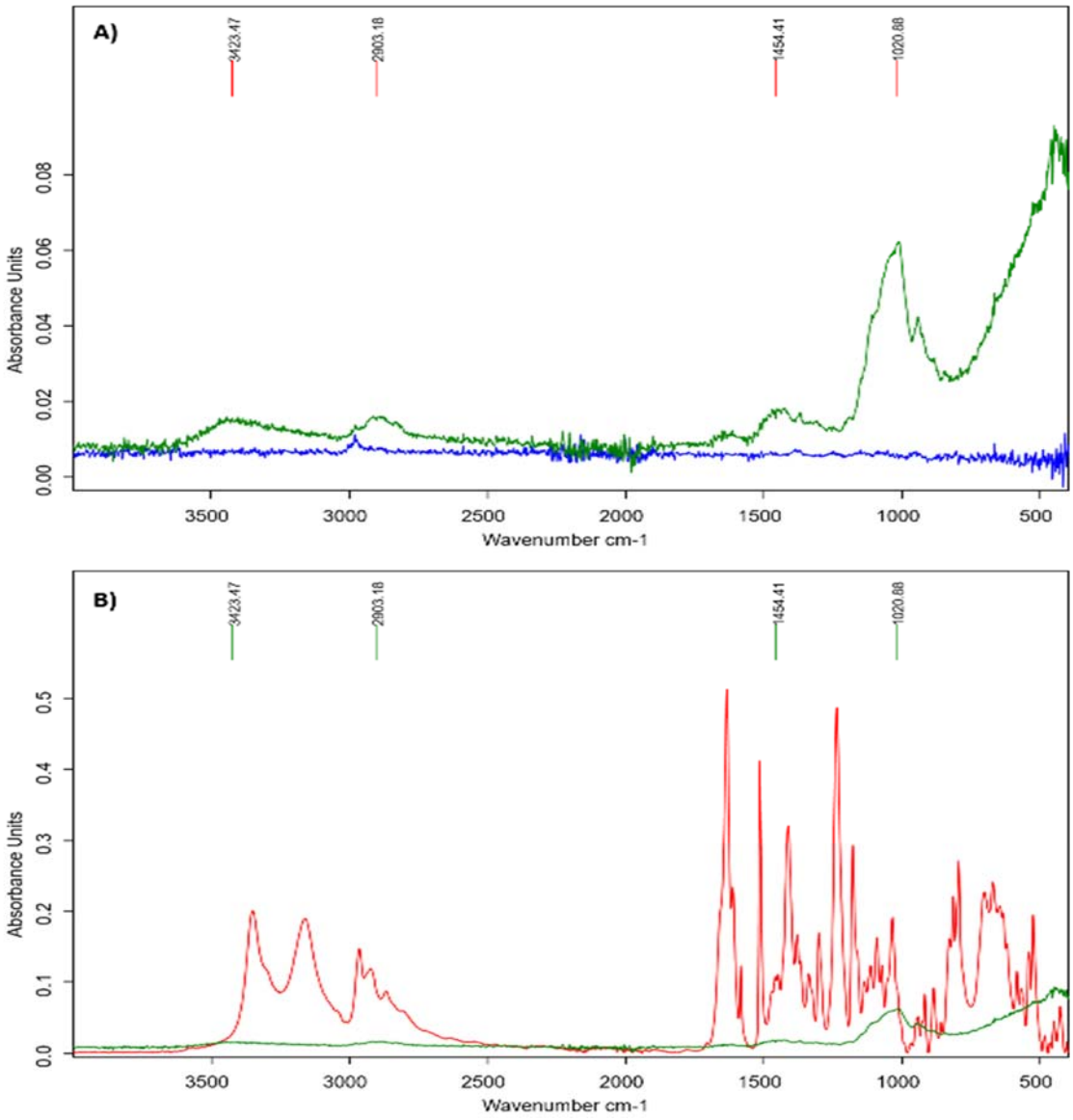

Figure 5.13: A) ATR FTIR Spectra of At/UK/3 edge (Blue) and At/UK/3 centre (Green), B) ATR FTIR Spectra of At/UK/3 centre (Green) and atenolol analytical reference (Red)

Figure 5.13 show differences between face and edge sampling for coated and non-coated tablets. The At/UK/3 sample is representative of atenolol tablets that are colour coated (orange). The spectra for the whole tablet of At/UK/3 (Figure 5.13A) shows differences between the centre and the edge of the tablet, this is due to the interaction of the tablet surface on the sampling area. The surface area of the tablet in contact with the sampling area is greater for the centre than when the edge is in contact, due to the shape of the tablet; this can be observed by the difference in intensity in the spectra. Figure 5.13B compares the whole tablet spectra to that of the atenolol analytical

reference, there are no characteristic peaks for atenolol visible and those that are present could be related to coating.

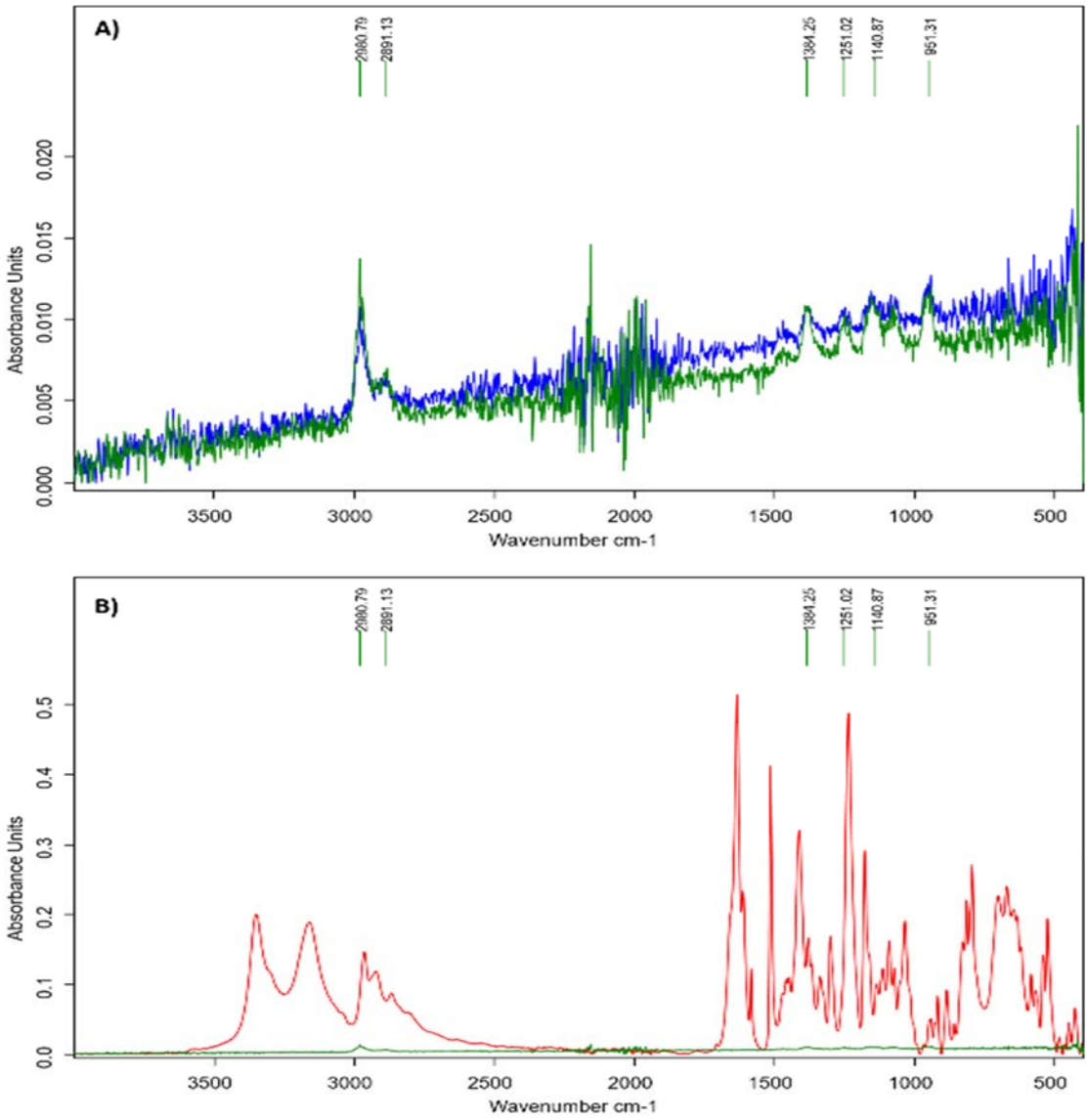

Figure 5.14: A) ATR FTIR Spectra of At/IND/6 edge (Blue) and At/IND/6 centre (Green), B) ATR FTIR Spectra of At/IND/6 centre (Green) and atenolol analytical reference (Red)

The At/IND/6 sample is representative of atenolol tablets that are non-coated and flat in shape. The spectra for the whole tablet of At/IND/6 (Figure 5.14A) shows little differences between the centre and the edge of the tablet, this is due to the shape of the tablet; as the tablet is flat all areas are in contact with the sampling area equally. Figure 5.14B compares the whole tablet spectra to that of the atenolol analytical reference, there are no clear characteristic peaks for atenolol, as the peak intensity is weak, this primarily due to ATR FTIR being a surface technique.

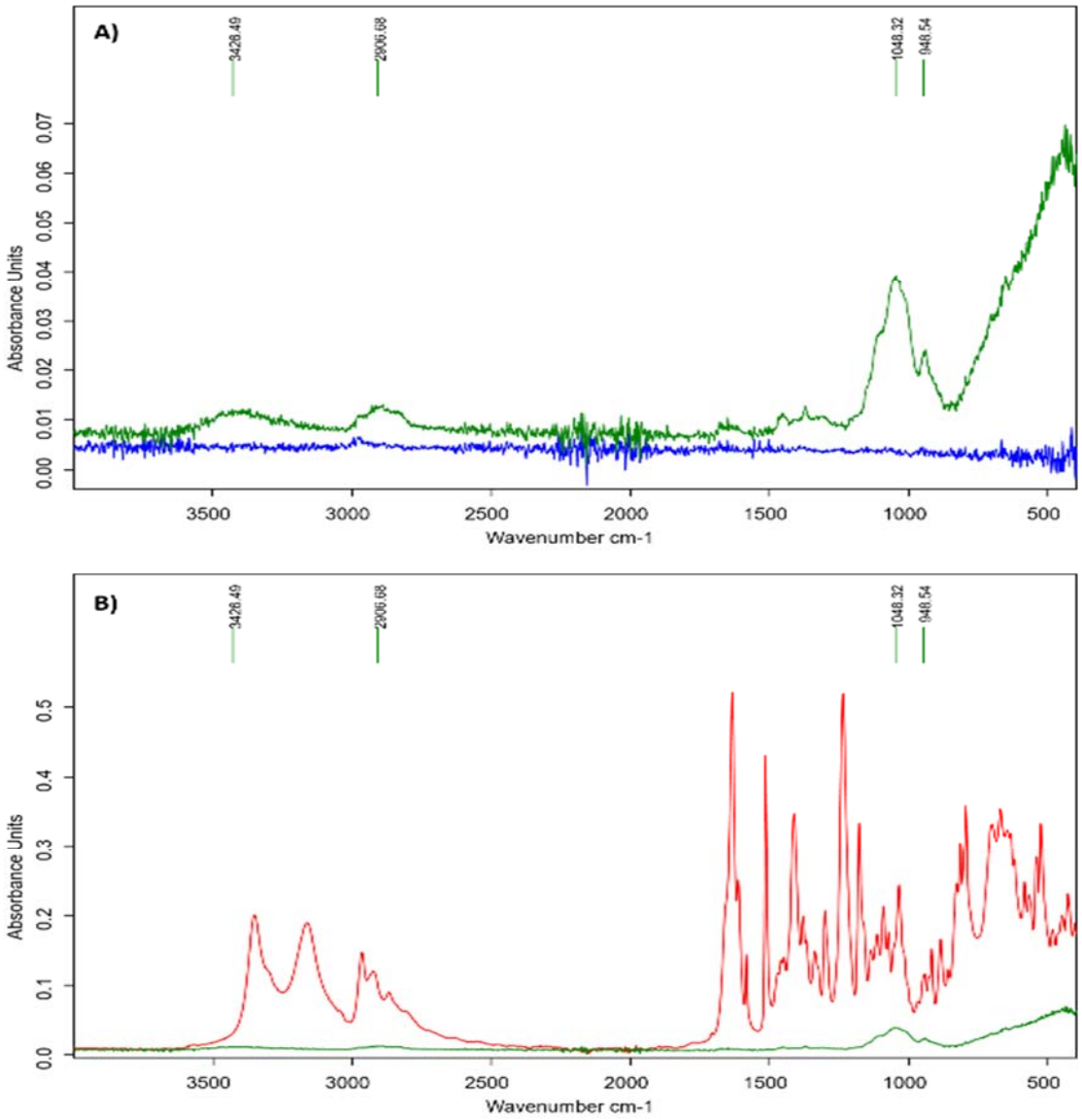

Figure 5.15: A) ATR FTIR Spectra of At/SA/1 edge (Blue) and At/SA/1 centre (Green), B) ATR FTIR Spectra of At/SA/1 centre (Green) and atenolol analytical reference (Red)

The spectra for the film coated atenolol sample (At/SA/1), showed similarities to the non-coated sample spectra, however there is some limited additional information from the surface of the film-coated sample but with insufficient signal intensity.

The spectra for the whole tablet of At/SA/1 (Figure 5.15A) shows differences between the centre and the edge of the tablet, this is due to the interaction of the tablet surface on the sampling area. Differences in peak intensity and resolution can be observed. Figure 5.15B compares the whole tablet spectra to that of the atenolol analytical reference, no characteristic peaks for atenolol are apparent and the peaks that are visible for At/SA/1 could be related to coating.

5.1.2.1.2 Metformin Hydrochloride Tablets

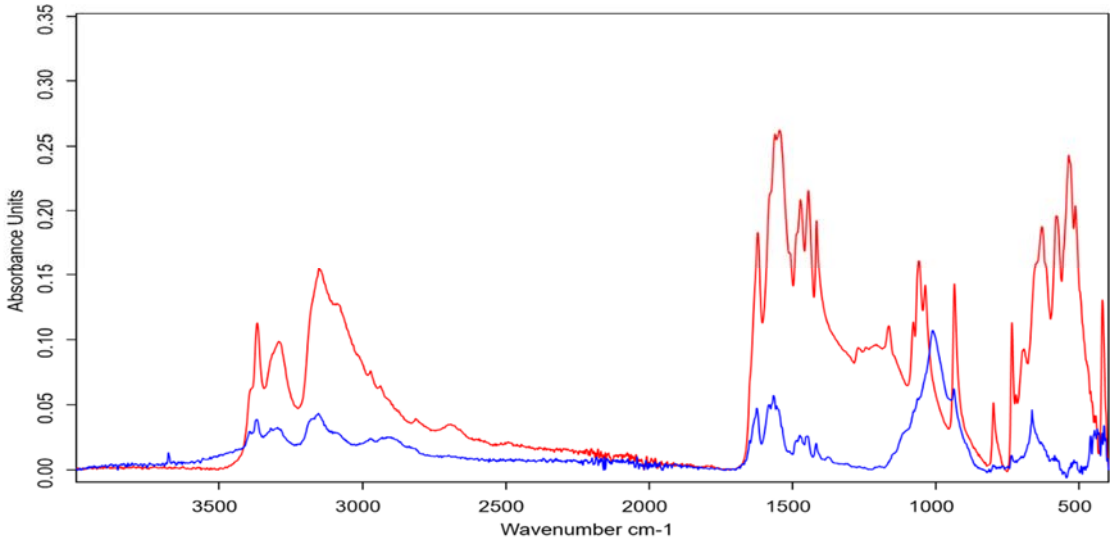

Figure 5.16: ATR FTIR Spectra of Met/UK/1 (Blue) and metformin hydrochloride analytical reference (Red)

The Met/UK/1 sample is representative of metformin hydrochloride tablets that is film coated and flat in shape. Figure 5.16 compares the whole tablet spectra to that of the metformin hydrochloride analytical reference, there are no clear characteristic peaks for metformin hydrochloride and any peaks observed are likely to be the coating, as ATR FTIR is primarily a surface technique.

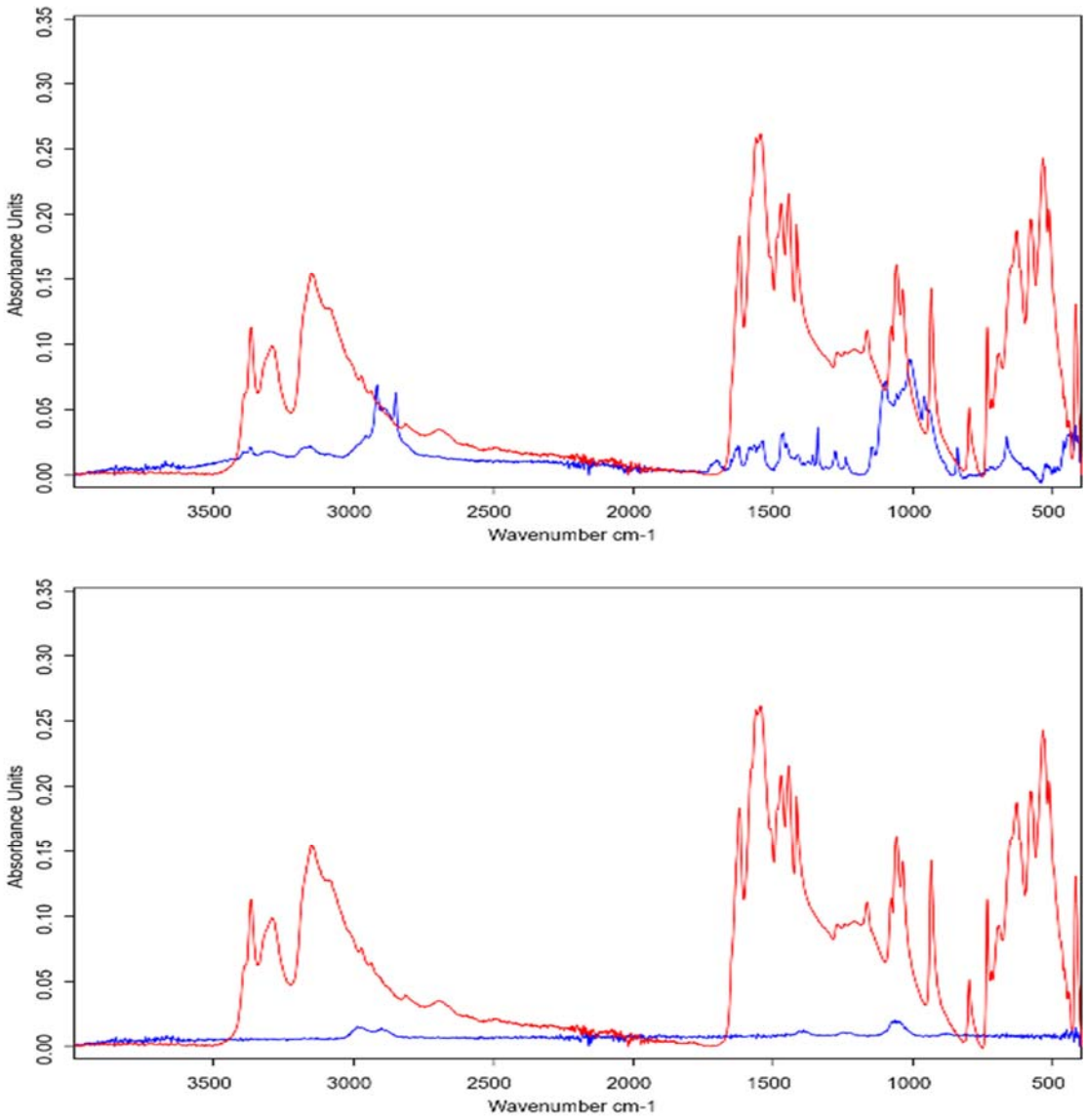

Figure 5.17: A) ATR FTIR Spectra of Met/IND/2 (Blue) and metformin hydrochloride analytical reference (Red), B) ATR FTIR Spectra of Met/IND/4 (Blue) and metformin hydrochloride analytical reference (Red)

Figure 5.17 compares the spectra of standard (Met/IND/2) and sustained/ extended release (Met/IND/4) tablets of the same dose (500mg) and the same manufacturer to that of the metformin hydrochloride analytical reference. There are no clear characteristic peaks for metformin hydrochloride observed for either sample and any peaks observed are likely to be the coating, as ATR FTIR is primarily a surface technique. The spectrum for Met/IND/2 (Figure 5.17A) showed few poorly resolved peaks at a low intensity, whereas for the spectrum for Met/IND/4 (Figure 5.17B) showed peaks at slightly lower intensity to that of the analytical reference.

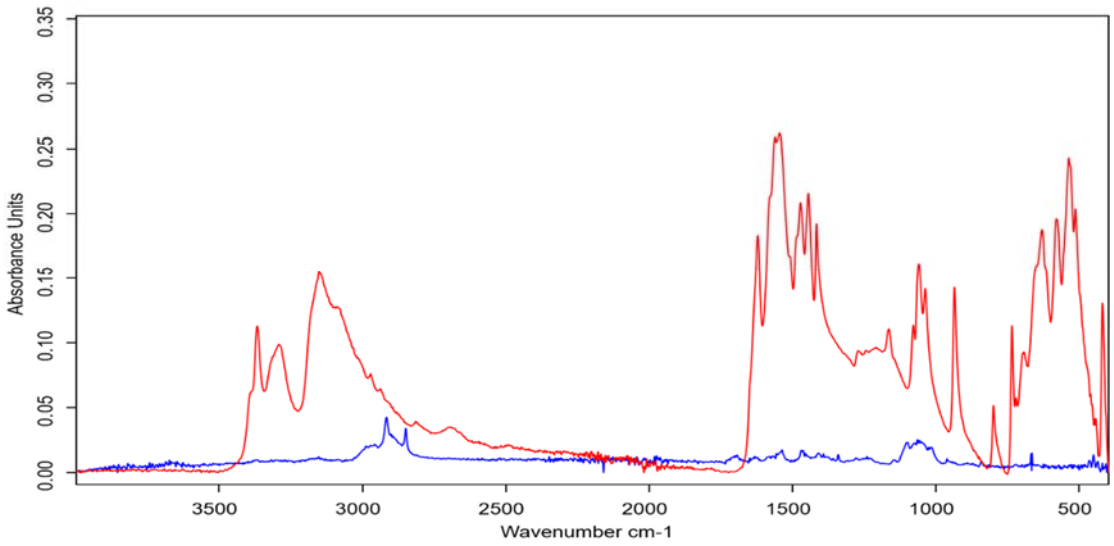

Figure 5.18: ATR FTIR Spectra of Met/IND/11 (Blue) and metformin hydrochloride analytical reference (Red)

The Met/IND/11 sample is representative of metformin hydrochloride tablets that contain another API. Figure 5.18 compares the whole tablet spectra to that of the metformin hydrochloride analytical reference, there are no clear characteristic peaks for metformin hydrochloride and any peaks observed are likely to be the coating, as ATR FTIR is primarily a surface technique.

5.1.2.1.3 Antimalarial Tablets

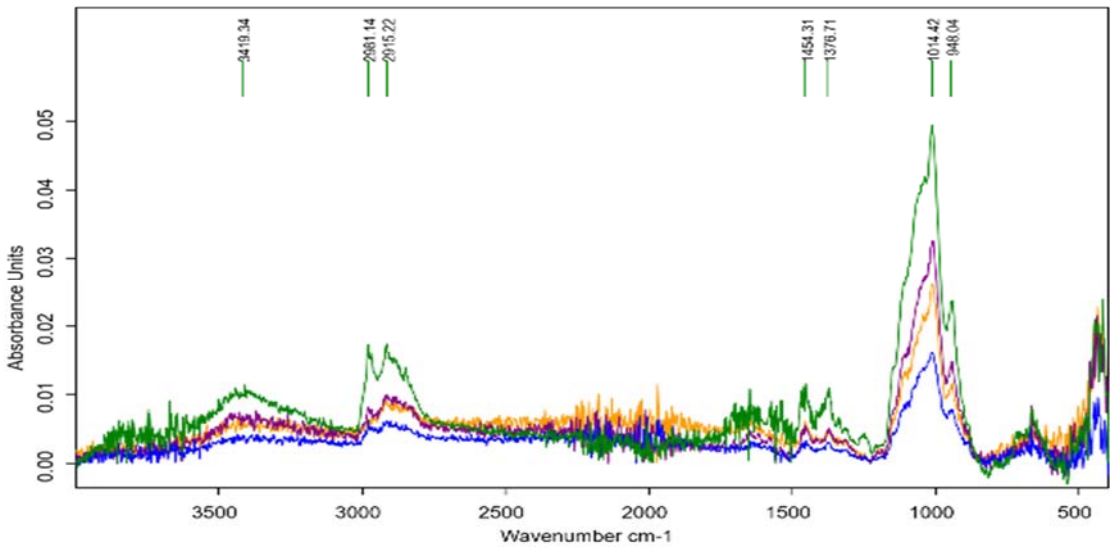

Figure 5.19: ATR FTIR Spectra of AM/ZIM/4 tablet 1 (Green), tablet 2 (Blue), tablet 3 (Purple) and tablet 4 (Yellow)

Results shown in Figure 5.19 are from one antimalarial AM/ZIM/4 sample, the API was identified as quinine sulphate. Multiple tablets were examined from this batch. AM/ZIM/4 contained four

tablets obtained from a hospital in Zimbabwe. The overlaid spectra for the four whole tablets of AM/ZIM/4 (Figure 5.19) shows differences between peak intensity and peak shape.

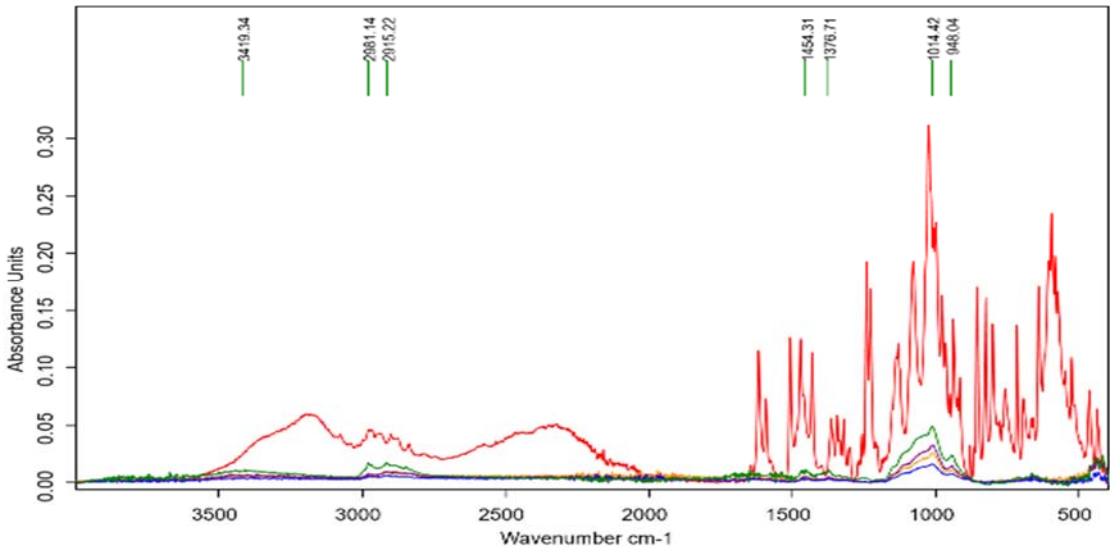

Figure 5.20: ATR FTIR Spectra of AM/ZIM/4 tablet 1 (Green), tablet 2 (Blue), tablet 3 (Purple), tablet 4 (Yellow) and quinine sulphate reference (Red)

Figure 5.20 compares the whole tablet spectra to that of the quinine sulphate reference, there are some characteristic peak shapes for quinine sulphate though these are hard to distinguish. There is certainly evidence of intensity variation due to either differences in the API concentration or differences on surface contact on the ATR instrument.

5.1.2.2 *Raman Spectroscopy*

5.1.2.2.1 Atenolol Tablets

Whole atenolol tablets were analysed, as Raman spectroscopy penetrates beneath the surface of the sample.

5.1.2.2.1.1 UK Tablets

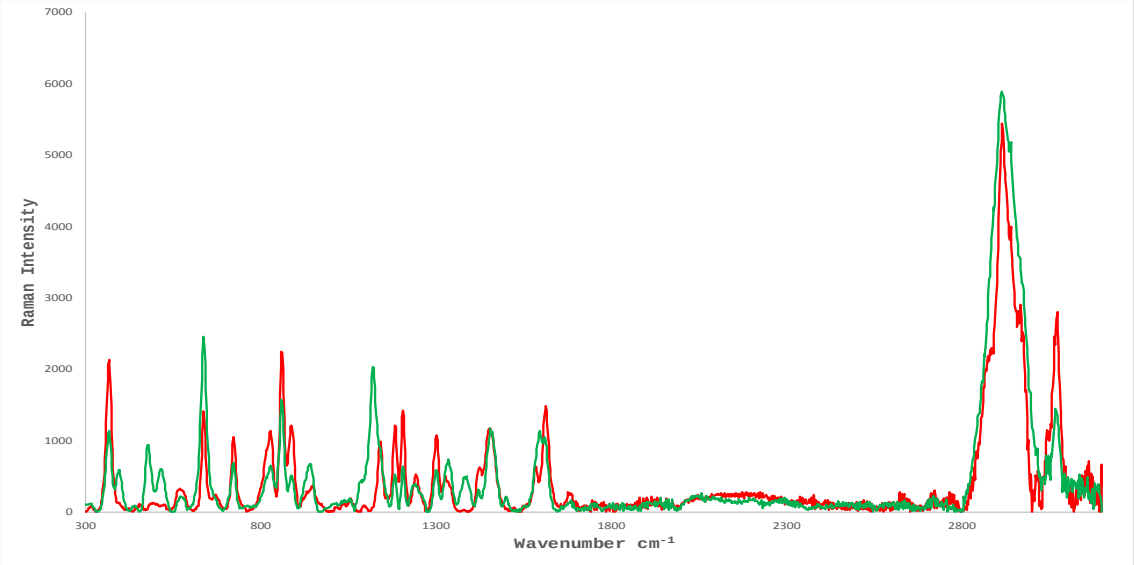

Figure 5.21: Raman Spectra of At/UK/3, BRAVO (Green) and atenolol analytical reference, BRAVO (Red)

The spectrum for the whole tablet of At/UK/3 (Figure 5.21) exhibited characteristic peaks for atenolol at 368cm^{-1}, 637cm^{-1}, 828cm^{-1}, 1187cm^{-1}, 1209cm^{-1}, 1301cm^{-1}, 1423cm^{-1}, 1451cm^{-1}, 1615cm^{-1}, 1680cm^{-1}, 2916cm^{-1} and 3072cm^{-1}. The spectrum also shows similarities with the atenolol analytical reference, however additional peaks at 480cm^{-1}, 520cm^{-1} and 1122cm^{-1} are present. These additional peaks could be excipient related, using the excipient library created for this work, it is possible to infer that croscarmellose sodium is present in this formulation. The Raman spectrum for At/UK/3 was able to confirm the presence of the API unlike the ATR FTIR spectrum for a whole tablet.

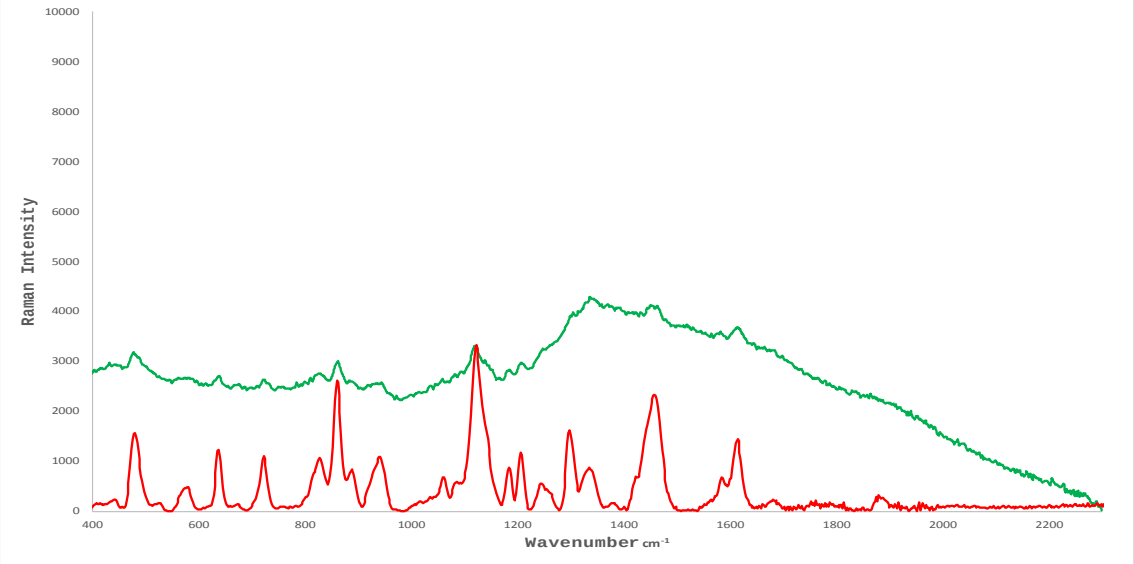

Figure 5.22: Raman spectra for At/UK/2 using BRAVO (Red) and MIRA-3 (Green) handheld spectrometers

Spectra for AT/UK/1-3 and At/UK/4-6 confirmed the presence of atenolol, as the spectra showed characteristic peaks for atenolol. The spectrum obtained using the MIRA-3 was heavily affected by fluorescence. Figure 5.22 compares the spectra obtained using the two different spectrometers (BRAVO and MIRA-3), with the MIRA-3 spectrum showing signs of fluorescence and poorly resolved peaks. Pharmaceutical samples often fluoresce, the amount and type of fluorescence is dependent on the material analysed and laser wavelength (Renishaw, 2015), this type of sample could promote fluorescence therefore swamping any underlying Raman spectrum and masking the signal (Horiba, 2017), as seen with the MIRA-3 spectrum. The spectrum for the FORAM-2 is not included as it is similar to the BRAVO spectrum.

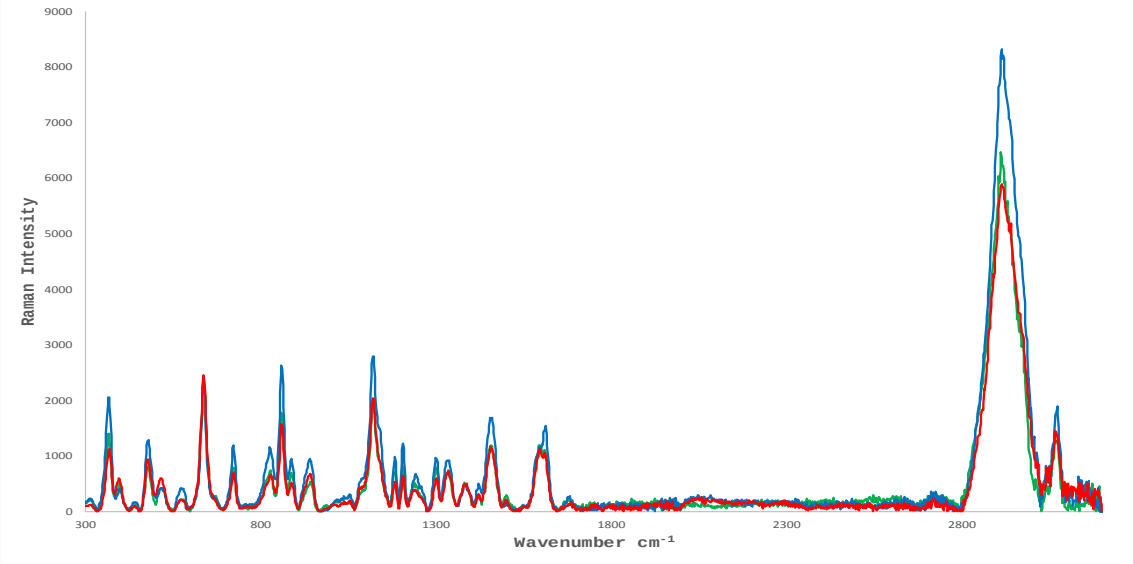

Figure 5.23: Raman spectra for At/UK/1 (Green), At/UK/2 (Blue) and At/UK/3 (Red) using the BRAVO spectrometer

The spectra for the different dosage levels (25mg, 50mg and 100mg) for both manufacturers showed good correlation of peak position, but little change in peak intensity in relation to the four-fold change in dosage (Figure 5.23).

5.1.2.2.1.2 Indian Tablets

All of the tablets that were analysed from India (At/IND/1 - 2, At/IND/4 – 16 and At/IND/18) confirmed the presence of atenolol using the BRAVO and FORAM spectrometers.

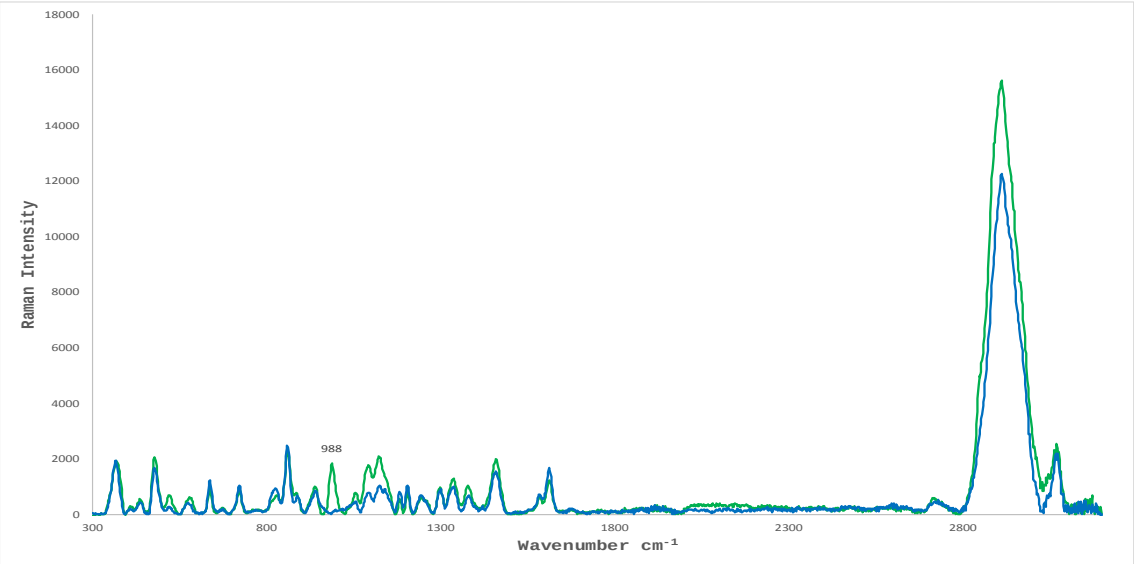

Figure 5.24: Raman Spectra of At/IND/1 25mg (Green) and At/IND/5 50mg (Blue) tablets using the BRAVO spectrometer

The spectra showed good agreement when comparing the different 25mg tablets together and the various 50mg tablets. This could indicate that the 25mg tablets were all manufactured using the same formulation and any variation in signal intensity could be attributed to matrix effects. These matrix effects are most likely caused by excipients within the sample, or the particle size of the API. Differences between the 25mg and 50mg formulations for At/IND/1 and At/IND/5 (Figure 5.24), indicates the presence of a different excipient used in the formulation of the 25mg tablet. The 25mg spectra all contained a peak at 988cm^{-1}, using the excipient library developed for this work; this peak can be attributed to dicalcium phosphate. This peak (988cm^{-1}) is absent in the UK 25mg formulations.

The spectra for the 50mg tablets analysed from different batches and manufacturers are in good agreement, suggesting that they were manufactured using the same formulation, with differing signal intensities attributed to matrix effects.

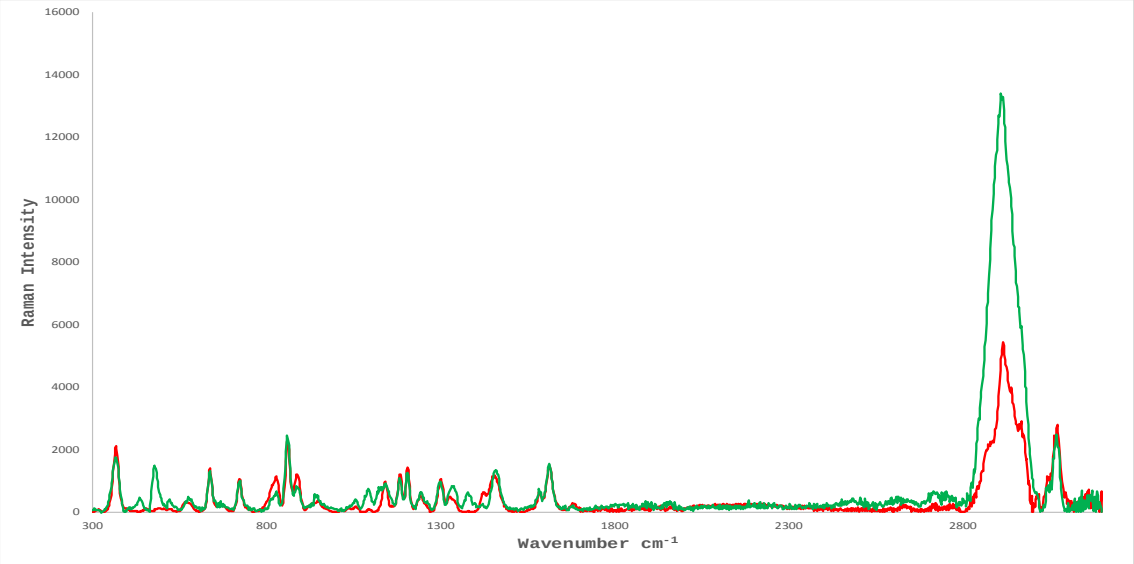

Figure 5.25: Raman Spectra of At/IND/6, BRAVO (Green) and atenolol analytical reference, BRAVO (Red)

The spectrum for the whole tablet of At/IND/6 (Figure 5.25) exhibited characteristic peaks for atenolol, however additional peaks at 476cm^{-1} and 1094cm^{-1} are present, and the peak at 2910cm^{-1} is a different shape and intensity to the reference. These additional peaks could be excipient related using the excipient library created for this work, it is possible to infer that MCC is present in this formulation, as MCC exhibits peaks at 1094cm^{-1} and 2894cm^{-1}. The Raman spectrum for At/IND/6 was able to confirm the presence of the API within a whole tablet unlike the ATR FTIR spectrum.

5.1.2.2.1.3 Pakistani Tablets

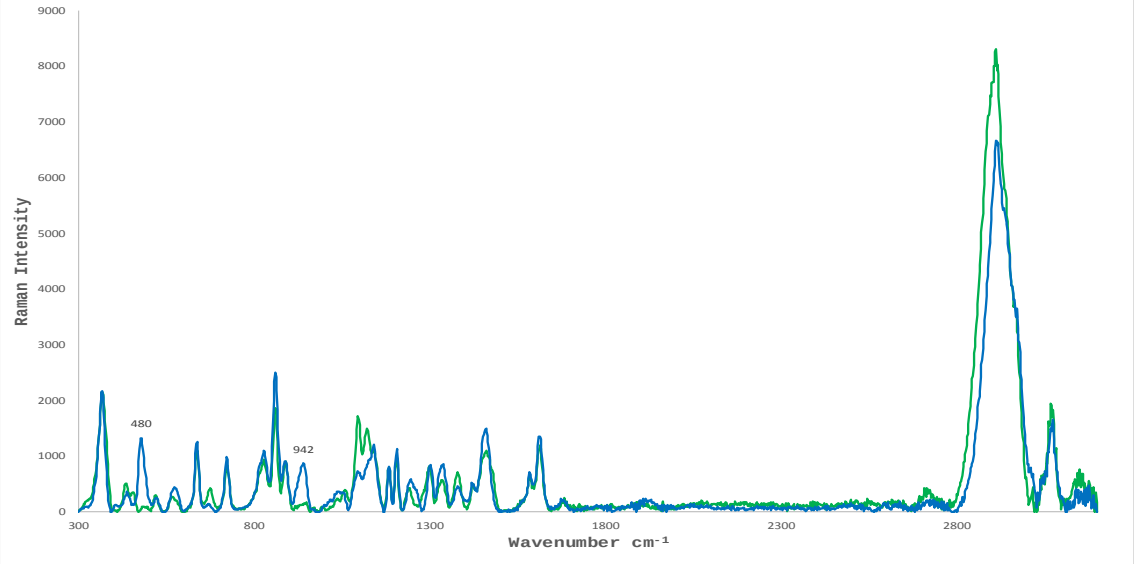

Figure 5.26: Raman Spectra of At/PAK/1 (Green) and At/PAK/2 (Blue) using the BRAVO spectrometer

The spectra for tablets At/PAK/1 and At/PAK/2 confirmed the presence of atenolol. There are some similarities between the spectra (Figure 5.26), but there are some clear differences indicating the use of different excipients in the formulation process. In particular peaks at 940cm^{-1} and 479cm^{-1} for the At/PAK/2 tablet. This could indicate the presence of starch or sodium starch glycolate, further work is required to confirm this.

5.1.2.2.1.4 Saudi Arabian Tablets

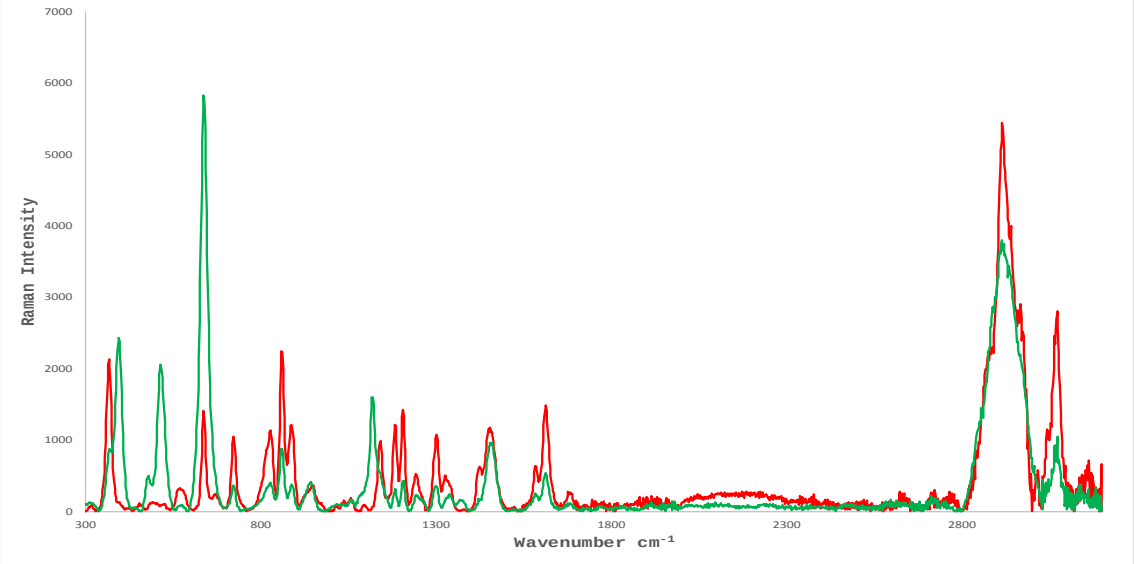

Figure 5.27: Raman Spectra of At/SA/1, BRAVO (Green) and atenolol analytical reference, BRAVO (Red)

The spectra for the two different manufacturers for the Saudi Arabian tablets (At/SA/1 & 2 and At/SA/3-5) confirmed the presence of atenolol.

The spectrum for the whole tablet of At/SA/1 (Figure 5.27) exhibited characteristic peaks for atenolol, however additional peaks at 396cm^{-1}, 516cm^{-1}, 638cm^{-1} and 1460cm^{-1} are present, and the peak at 1118cm^{-1} is a different shape to the reference. These additional peaks (396cm^{-1}, 516cm^{-1} , 638cm^{-1} and 1460cm^{-1}) could be excipient related, using the excipient library created for this work, it is possible to infer that titanium dioxide is present in this formulation, most likely in the coating, as well as starch. The spectrum for At/SA/2 is not shown, as it is identical to the one shown for At/SA/1.

5.1.2.2.1.5 Nepalese Samples

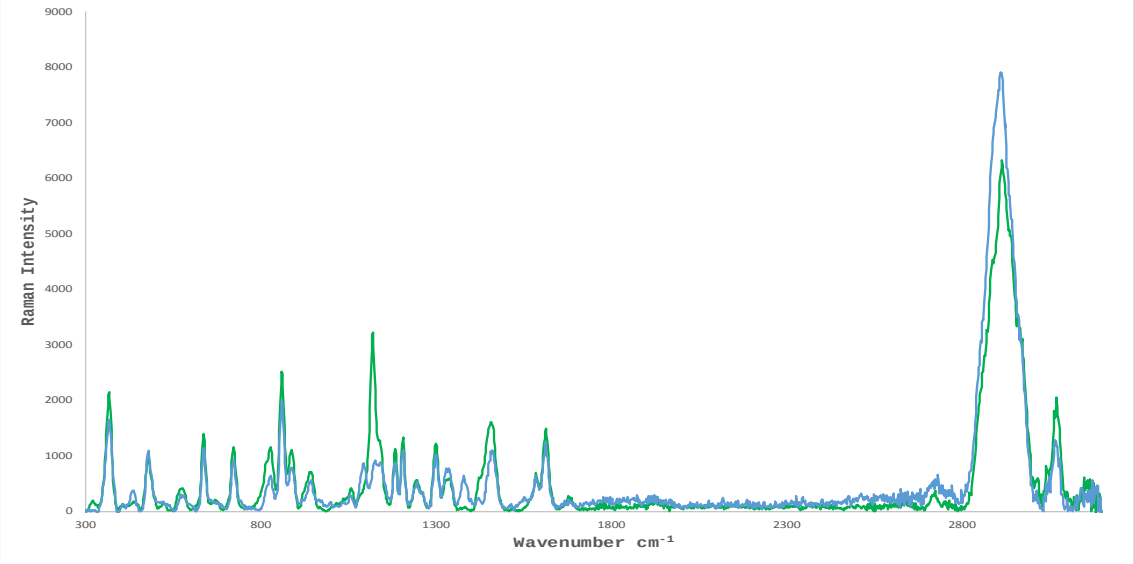

Figure 5.28: Raman Spectra of AT/NEP/1 (Green) and At/NEP/2 (Blue) tablets using the BRAVO spectrometer

All three samples analysed from Nepal indicate that atenolol was present in the tablet samples. The spectra for At/NEP/2 and At/NEP/3 are from the same manufacturer and are in good agreement, with a slight difference in peak intensity (~2911cm^{-1}); this difference may be attributed to matrix effects. The At/NEP/1 spectrum is different to the AT/NEP/2 and At/NEP/3 spectra, suggesting that they are a different formulation (Figure 5.28). The more intense peak for At/NEP/1 at 1120cm^{-1} could indicate the presence of starch, as listed in the patient information leaflet. The excipients for At/NEP/2 and At/NEP/3 were unavailable, further work is required to determine if the inclusion of different excipients is the sole reason for the difference between At/NEP/1 and At/NEP/2 & 3.

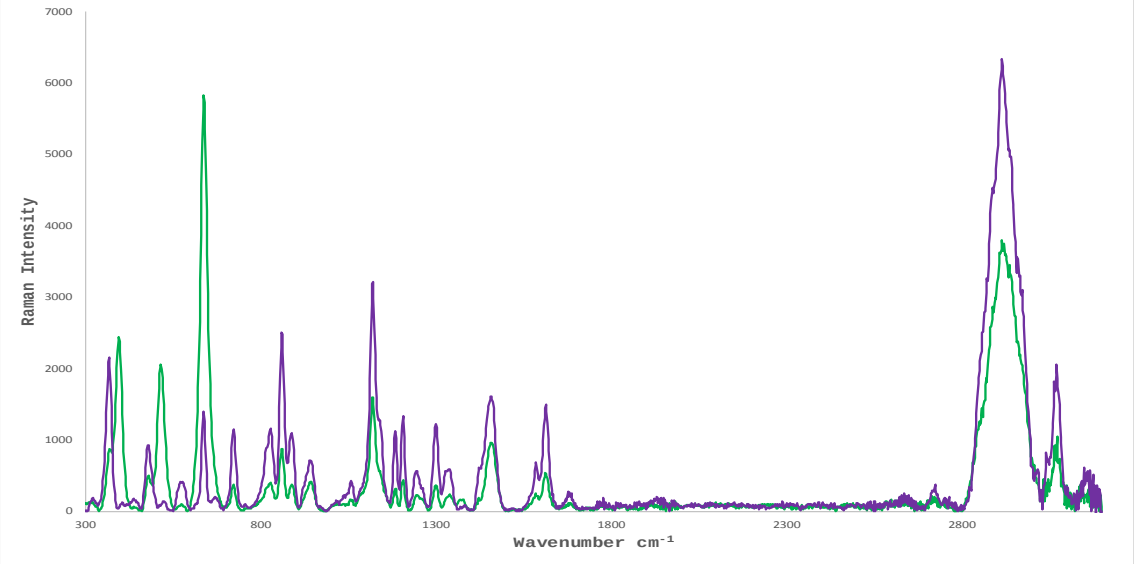

Figure 5.29: Raman Spectra of At/SA/1 (Green) and At/NEP/1 (Purple) using the BRAVO spectrometer

However, when the At/NEP/1 sample is compared to that of the same manufacturer from Saudi Arabia (At/SA/1) (Figure 5.29), the spectra are not in good agreement. The Nepalese sample spectrum shows similar peaks to those in the Saudi Arabian samples, but at different intensities. The Nepalese sample also shows some peaks at different positions (368cm^{-1} and 480cm^{-1}) and the addition of a peak at 576cm^{-1}, indicating the possibility of titanium dioxide in the coating.

5.1.2.2.2 Metformin Hydrochloride

Whole tablet spectra for metformin hydrochloride samples were obtained using the benchtop FORAM-2 spectrometer.

5.1.2.2.2.1 UK Tablets

The Raman spectra using the FORAM-2 spectrometer confirmed the presence of metformin hydrochloride in the 500mg and 850mg formulations, as characteristic peaks were observed in the spectra.

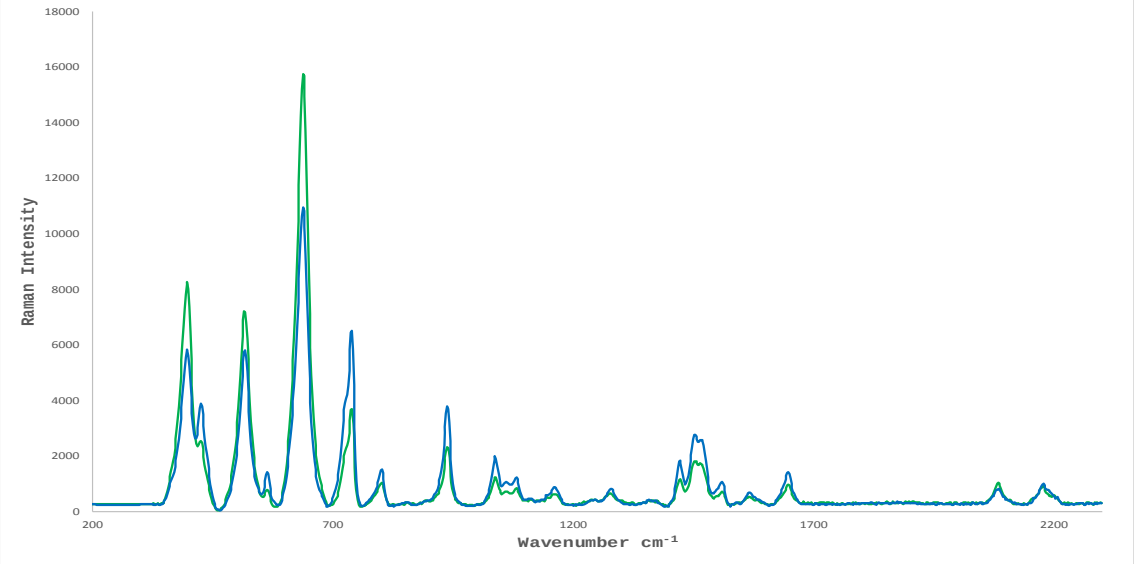

Figure 5.30: Raman Spectra of Met/UK/1 (Green) and Met/UK/2 (Blue) using the FORAM-2 spectrometer

The spectra showed good agreement for the different dosages tablets (Figure 5.30), with the only differences in signal intensity; this may be due to the different levels of API in the tablet. There are no extra peaks observed from excipients when compared to the reference standard spectrum; this may be due to the metformin hydrochloride tablets containing a high percentage concentration of API in relation to the tablet weight (~85%).

5.1.2.2.2.2 Indian Tablets

All the tablets from India confirmed the presence of metformin hydrochloride, there was generally good agreement between the spectra. Using the FORAM-2 spectrometer all the peaks were clearly resolved and matched the analytical reference spectrum.

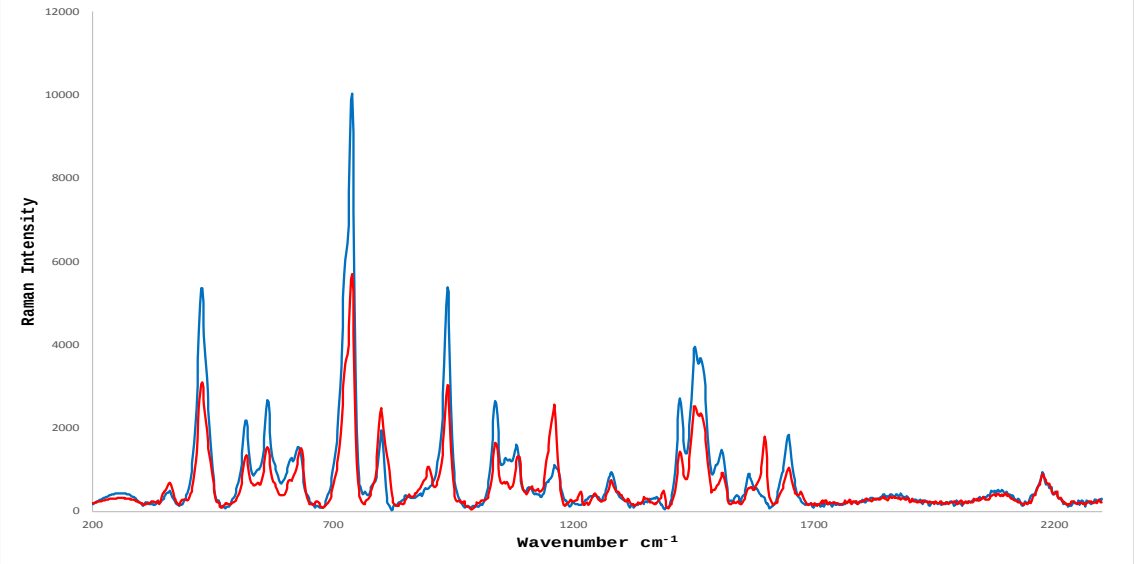

Figure 5.31: Raman Spectra for Met/IND/6 (Blue) and Met/IND/11 (Red) tablets using the FORAM-2 spectrometer

Some of the tablet samples analysed were stated to contain another API (Met/IND/6, - 8, Met/IND/11 – 14). No significant differences between the sample and analytical reference standard were observed between the spectra (Figure 5.31), as metformin hydrochloride is present at a high concentration compared to the other API's. This suggests that there may be a lower detection limit for mixtures of several APIs. This is investigated in the following chapter.

No differences were observed between the spectra for standard release and extended/sustained release, suggesting either little or no difference in the formulation of the tablets or the differences are not detectable because of the high API level.

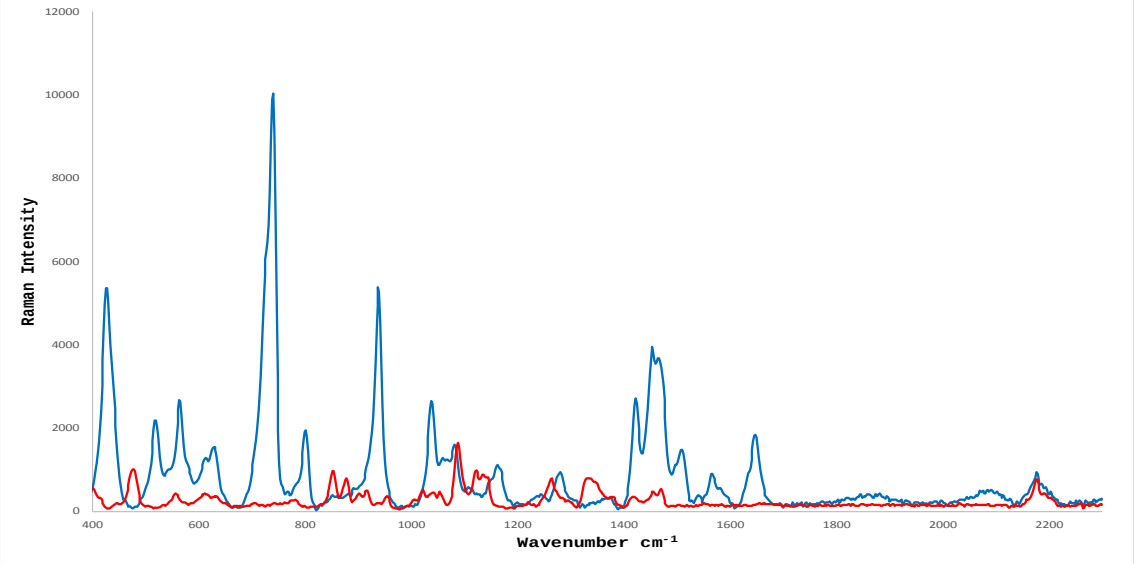

Figure 5.32: Raman Spectra of Met/IND/6 (Blue), score line (Red) using FORAM-2 spectrometer

Tablet formulations are usually assumed to be uniform within any coating material, but in some instances the appearance of the tablet indicates two apparently different material layers to be present. This situation occurs for some multiple API diabetes tablets and also some antimalarial tablets. In this study the sample Met/IND/6 is an example of this, there are clear differences in the spectra (Figure 5.32) for the two sides, with the non-score line face exhibiting characteristic peaks for metformin hydrochloride.

5.1.2.2.2.3 Saudi Arabian Tablets

The spectrum for the sample from Saudi Arabia confirmed the presence of metformin hydrochloride, as characteristic metformin hydrochloride peaks could be identified.

5.1.2.2.3 Antimalarial Tablets from Zimbabwe

Whole antimalarial tablets were analysed as per 5.1.2.1.2 using the benchtop system (FORAM-2). The AM/ZIM/4 sample set demonstrates variation within a batch.

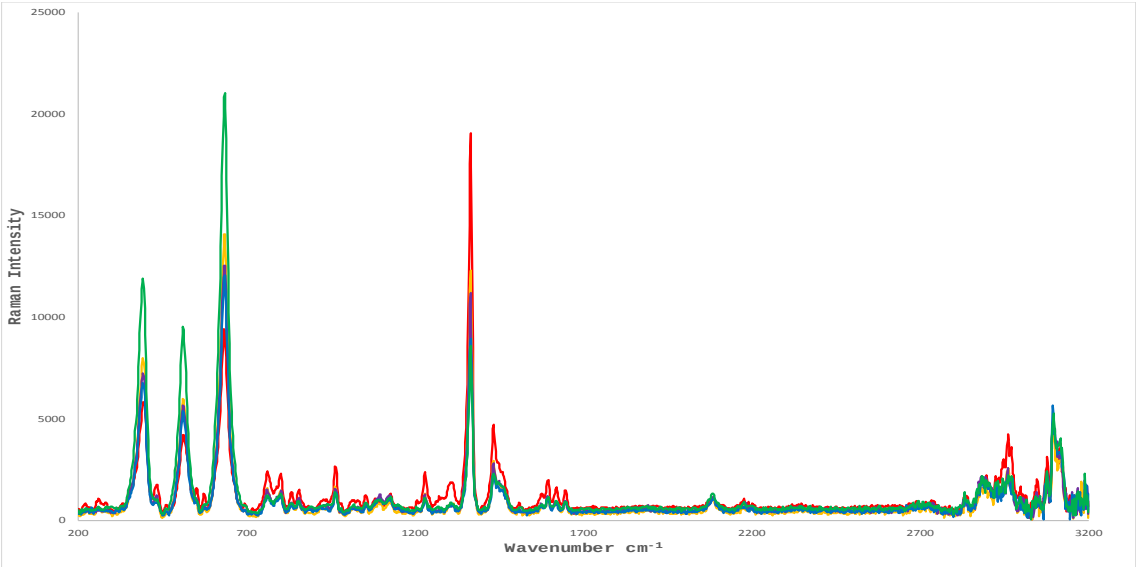

Figure 5.33: A) Raman spectra of AM/ZIM/4 tablet 1 (Green), tablet 2 (Blue), tablet 3 (Purple), tablet 4 (Yellow) and tablet 5 (Red)

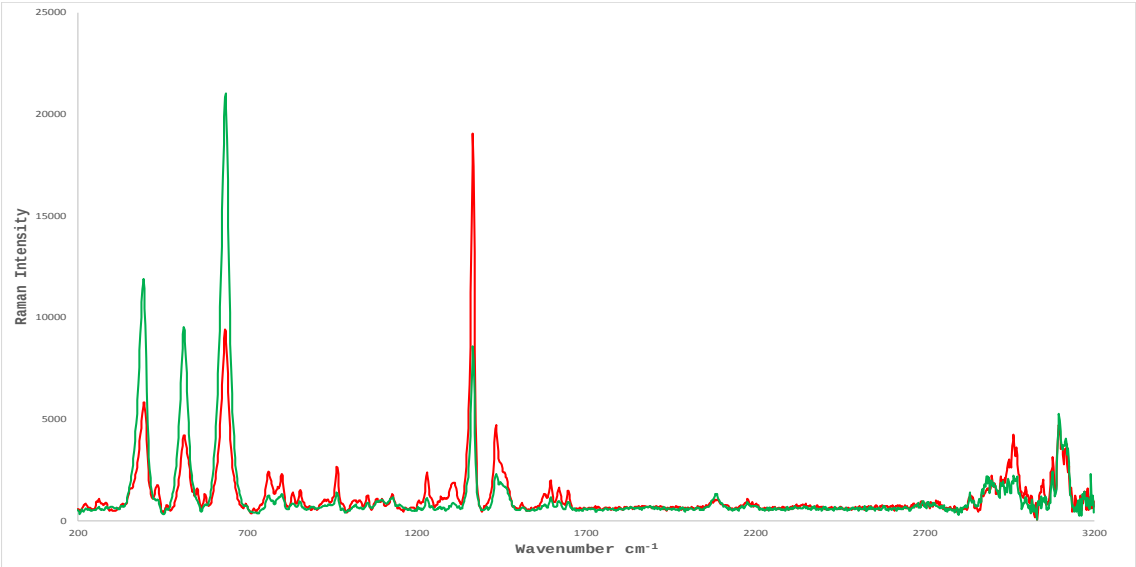

Figure 5.34: Raman spectra of AM/ZIM/4 tablet 1 (Green), and tablet 5 (Red)

The spectra for the whole tablet of AM/ZIM/4 (Figure 5.33) shows differences between peak intensity and peak shape. Figure 5.34 compares tablet 1 and 5, there are differences between peak intensity and ratio of peaks for the two spectra. The peaks at 392cm^{-1}, 512cm^{-1} and 636cm^{-1}, are indicative of titanium dioxide and the peak at 1366cm^{-1} is characteristic of quinine sulphate. The spectrum for tablet 1 indicates a higher concentration of titanium dioxide than quinine sulphate, whereas the spectrum for tablet 5 shows a higher concentration of quinine sulphate, with different levels in tablets 2, 3 and 4. This is a clear example of poor quality control during the preparation

of the tablets. These differences may be due to surface coatings and the distribution of API and excipients within the tablet or could possibly indicate a difference in level of API within the tablet.

5.1.2.3 *Discussion*

It is clear from the data obtained that only Raman analyses can provide consistently meaningful data from the investigation of whole tablets. ATR FTIR can in some circumstances, provide information but this is dependent on adequate surface contact between the sample and the sampling region. In short, it is sample/tablet shape dependent and is therefore unsuitable for a rapid identification system for whole tablets.

The Raman spectra for supposedly the same tablets from a range of different sources have shown, in general the same peaks to be present, indicative of the API or so called 'good Raman absorbers' within the excipients. Furthermore, this work presents evidence of the significant changes the Raman Effect can produce when looking at tablets from the same batch. The AM/ZIM/4 series of results are particularly informative and one explanation may be poor QC on the titanium dioxide coating on individual tablets. The coating will affect the level of the Raman Effect and hence the signal observed.

5.1.3 Powered Tablet Sample Studies

Whole tablets were powdered to give homogenous samples that would provide sufficient contact to obtain meaningful and reproducible data for both ATR and Raman investigations.

5.1.3.1 *ATR FTIR Spectroscopy*

5.1.3.1.1.1 UK Atenolol Tablets

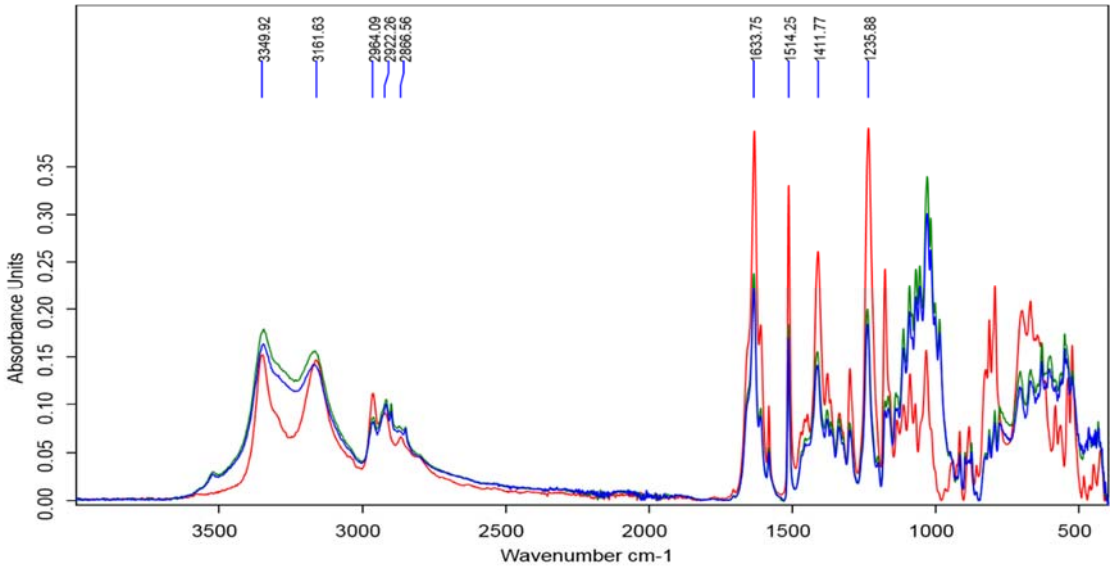

Figure 5.35: ATR FTIR Spectra of At/UK/2 (pre-2016) (50mg) (Blue) and At/UK/3 (pre-2016) (100mg) (Green) and atenolol analytical reference (Red)

The powdered samples analysed produce much better spectra as expected and the spectrum for the At/UK/2 50mg and At/UK/3 100mg (pre-2016) samples (Figure 5.35) exhibited the characteristic peaks for atenolol. The first thing to note is that the peak intensities for the two tablet samples are virtually identical, despite a factor of 2 in dosage level. This suggests that the size of the tablet increases with dose and the relative concentrations within the tablet remains the same. The peak shape for the aliphatic CH (2954cm^{-1} & 2922cm^{-1}) and carbonyl (1634cm^{-1}) are not identical to those of the atenolol reference spectra. Other excipients could exhibit peaks in the same regions, therefore slightly altering the peak shape. The aromatic peak at 1514cm^{-1} is not masked by any excipients present in this tablet formulation and is clearly identifiable, as is the peak at 1236cm^{-1}.

From looking at the spectra excipients can be identified within the sample, using the excipient reference spectra obtained. Maize starch exhibits a hump in the range of 3500– 2500cm^{-1}, which could account for the hydroxyl and amine peaks being raised in the spectra; maize starch also exhibits peaks at 1200 – 1000cm^{-1} and the peak shape of the CH aliphatic group differing from the atenolol reference spectra. This is also consistent with the patient information leaflet contained within the packaging.

Overall none of the excipients used in the At/UK/2 sample (pre-2016) formulation exhibit peaks at 1514cm^{-1} and 1236cm^{-1}; clear sharp peaks can be observed and are not masked nor is the peak shape altered by other excipients, unlike other characteristic atenolol peaks. Whilst we can still

detect the hydroxyl, amine and aliphatic CH peaks, the peak shape is slightly different and a range of excipients exhibit peaks in these regions. Thus, enabling the aromatic ring and amide peaks to be used as a marker to detect atenolol in the tablet and could be used to quantify the amount of atenolol present.

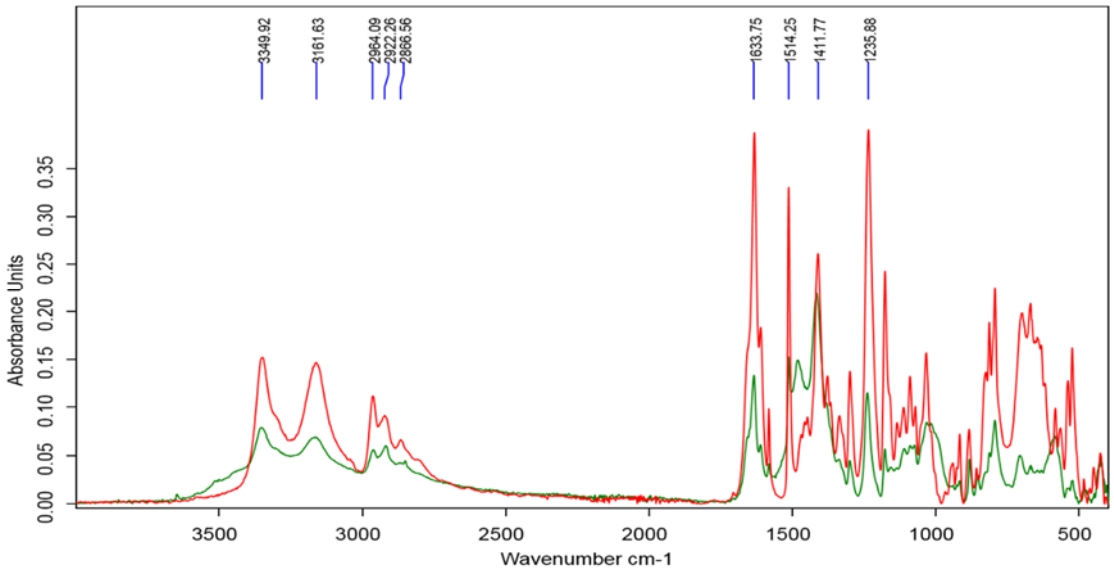

Figure 5.36: ATR FTIR Spectra of At/UK/1 (25 mg) (Green) overlaid with atenolol analytical reference (Red)

The spectrum for the At/UK/1 25mg sample (Figure 5.36) exhibited the characteristic peaks for atenolol, however when the spectra is compared; the aromatic ring (1514cm^{-1}), carbonyl (1634cm^{-1}) and nitro compound (1412cm^{-1}) are not identical to those of the atenolol reference spectra.

Other excipients could exhibit peaks in the same regions, therefore slightly altering the peak shape. The aromatic ring peak at 1514cm^{-1} is masked by magnesium carbonate; no other excipients produce a peak at this wavenumber. The amide peak (1236cm^{-1}) is not masked by any excipients and a clear sharp peak can be observed. Excipients can be identified within the sample using the excipient reference spectra obtained, with at least one excipient falling in the range of the characteristic atenolol peaks, as detailed in Table 5.1.

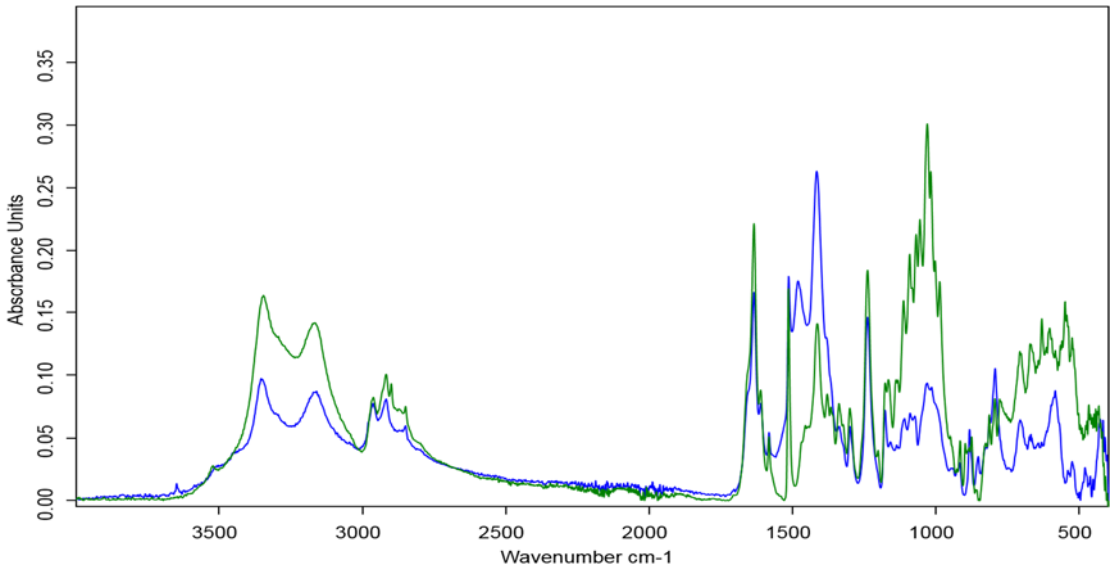

Figure 5.37: ATR FTIR spectra of At/UK/2 pre-2016 (Green) and At/UK/2 post-2016 (Blue)

The formulation for atenolol tablets At/UK/1, At/UK/2 and At/UK/3 appears to have changed post 2016. Figure 5.37 shows At/UK/2 pre-2016 overlaid with At/UK/2 post-2016 formulation. This is important to note when analysing spectra to identify SF tablets that formulations for the same manufacturer may change without notice. The once visible aromatic ring peak (1514cm^{-1}) is now masked by magnesium carbonate within the formulation; this change can be confirmed by analysing the spectra using the excipient library.

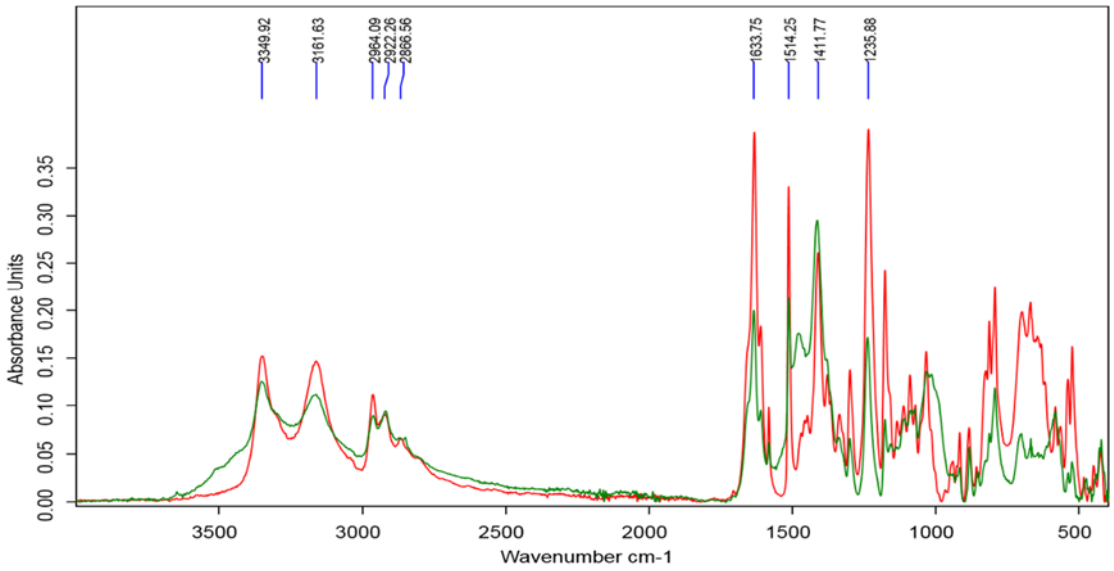

Figure 5.38: ATR FTIR Spectra of At/UK/4 (25 mg) (Green) overlaid with atenolol analytical reference (Red)

The spectra for the AT/UK/4 (Figure 5.38) exhibited the characteristic peaks for atenolol; however, the aromatic ring (1514cm^{-1}), carbonyl (1634cm^{-1}) and nitro compound (1412cm^{-1}) are

not identical to those of the atenolol analytical reference. Other excipients could exhibit peaks in the same regions, therefore slightly altering the peak shape. The aromatic ring peak at 1514cm^{-1} is masked by magnesium carbonate, all other excipients in this tablet are absent at this wavenumber. The amide peak (1236cm^{-1}) is not masked by any excipients and a clear sharp peak can be observed. Excipients can be identified within the sample using the excipient reference spectra obtained. Magnesium carbonate exhibits a peak in the range of 1550–1000cm^{-1}, which masks the aromatic ring peak. A clear sharp peak can be identified at 1236cm^{-1}, enabling this peak to be used as a marker to detect atenolol in the tablet and possibly to quantify the amount of atenolol present.

The spectra for the At/UK/4, At/UK/5, At/UK/6 and At/UK/7 tablets are identical and the same points can be considered. It is worth noting that the samples for At/UK/1 – 3; At/UK/4 - 6 and At/UK/7 are identical to one another indicating that they are of the same formulation.

5.1.3.1.1.2 Indian Atenolol Tablets

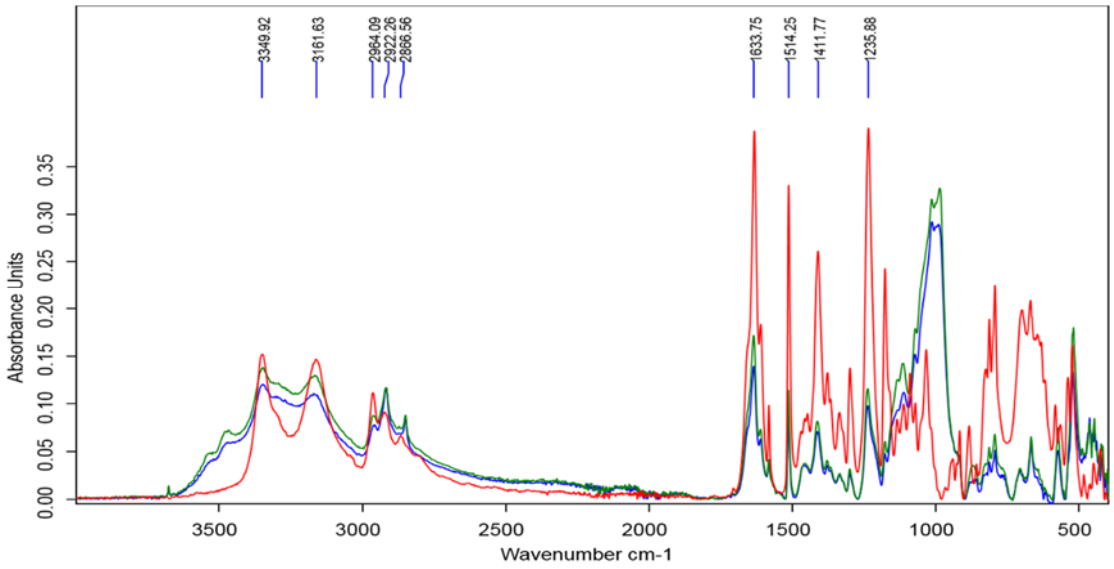

Figure 5.39: ATR FTIR Spectra of At/IND/1 (Green) and At/IND/2 (Blue) (25 mg) overlaid with atenolol analytical reference (Red)

The spectra for the At/IND/1 and At/IND/2 25mg tablets (Figure 5.39) are identical to one another and exhibited the characteristic peaks for atenolol; however, the hydroxyl, amine (3350cm^{-1} and 3152cm^{-1}) and aliphatic CH (2954cm^{-1} and 2922cm^{-1}) peaks are not identical to those of the atenolol analytical reference.

The excipient list for this tablet was unavailable, however using the excipient reference spectra; excipients can be identified within the sample. Maize starch exhibits a hump in the range of 3500–2500cm^{-1}, which could account for the hydroxyl and amine peaks being raised in the spectra. MCC can be identified from the spectra, with peaks at 1150 – 1000cm^{-1}. Overall none of the excipients used in the At/IND/1 and At/IND/2 tablet formulation exhibit some peaks at 1514cm^{-1} and 1236cm^{-1}. Clear sharp peaks can be observed and are not masked or the peak shape altered by other excipients, unlike other characteristic atenolol peaks.

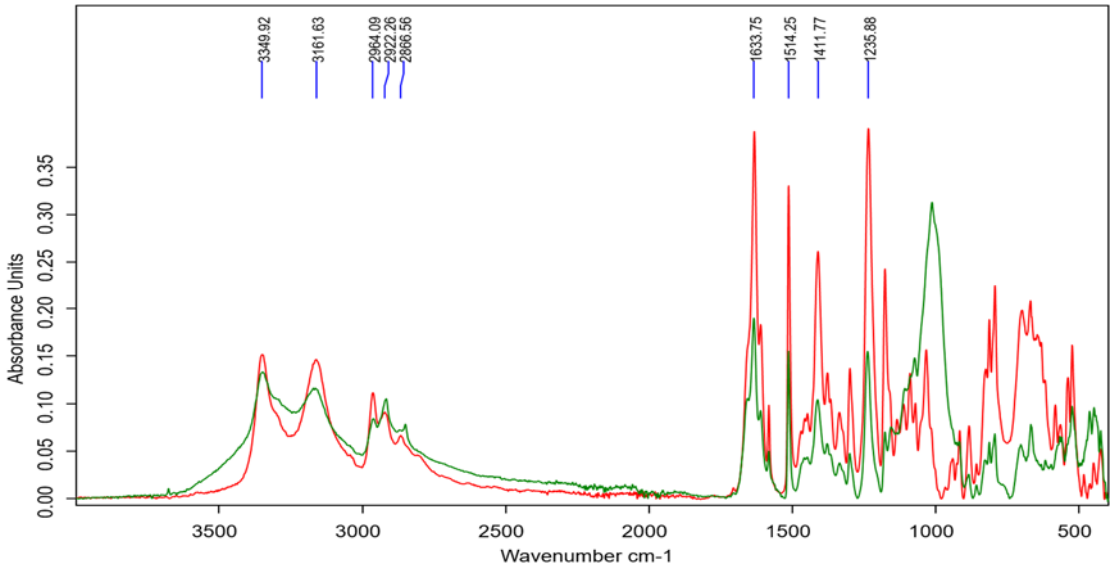

Figure 5.40: ATR FTIR spectrum of At/IND/5 (50mg)

The spectra for the At/IND/5 50mg tablet (Figure 5.40) exhibited the characteristic peaks for atenolol; however, the hydroxyl, amine (3350cm^{-1} and 3152cm^{-1}) and aliphatic CH (2954cm^{-1} and 2922cm^{-1}) peaks are not identical to those of the atenolol reference spectra. Other excipients could exhibit peaks in the same regions, therefore slightly altering the peak shape. The aromatic peak at 1515cm^{-1} and the amide peak at 1236cm^{-1} are not masked by any excipients present in this tablet formulation and are clearly identifiable.

Excipients can be identified within the sample using the excipient reference spectra obtained. Maize starch exhibits a hump in the range of 3500 – 2500cm^{-1}, which could account for the hydroxyl and amine peaks being raised in the spectra. MCC can be identified from the spectra, with peaks exhibited at 1150 – 1000 cm^{-1}. Comparing this spectrum with that of tablets from the same manufacturer, the spectra are identical. As the At/IND/5 – At/IND/10 tablets all confirm the presence of atenolol and appear to be the same formulation the spectra are included as an overlay in Figure 5.41

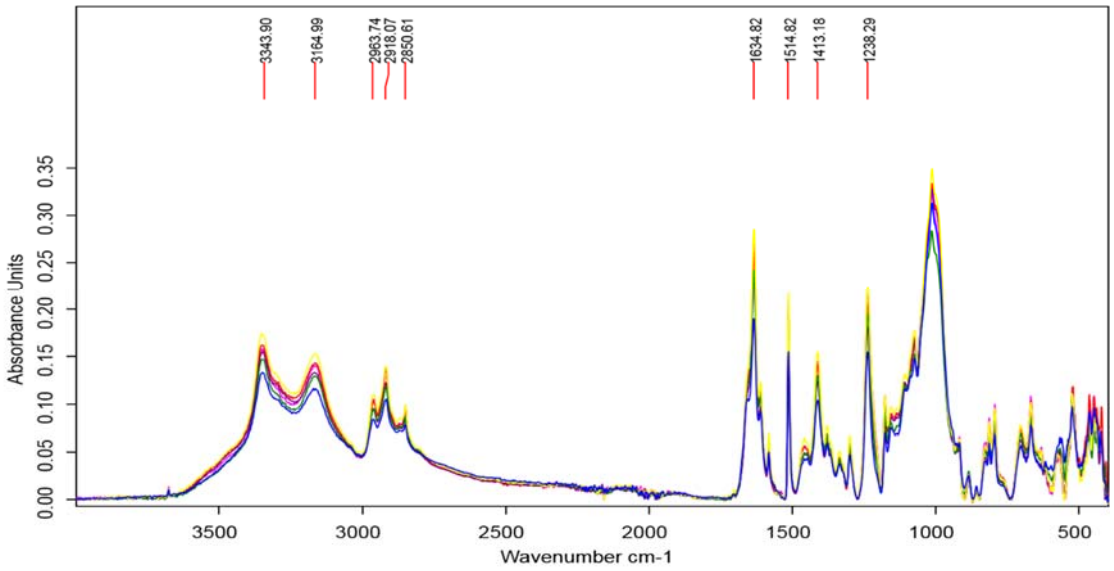

Figure 5.41: ATR FTIR spectra of At/IND/5 (Blue), At/IND/6 (Green), At/IND/7 (Purple), At/IND/8 (Yellow), At/IND/9 (Red) and At/IND/10 (Pink) Atenolol (50 mg)

The close agreement of these spectra demonstrate both the reproducibility of the analytical process and the good QC applied to the production of these tablets from different sources throughout India.

The analyses showed that all the Indian samples were represented by the data shown in either Figure 5.39 or Figure 5.40 and are as follows:

- Figure 5.39 – At/IND/1 – 4, At/IND/11 and At/IND/12
- Figure 5.40 – At/IND/5 – 10, At/IND/12 – 13, At/IND/15 – 16 and At/IND/18.

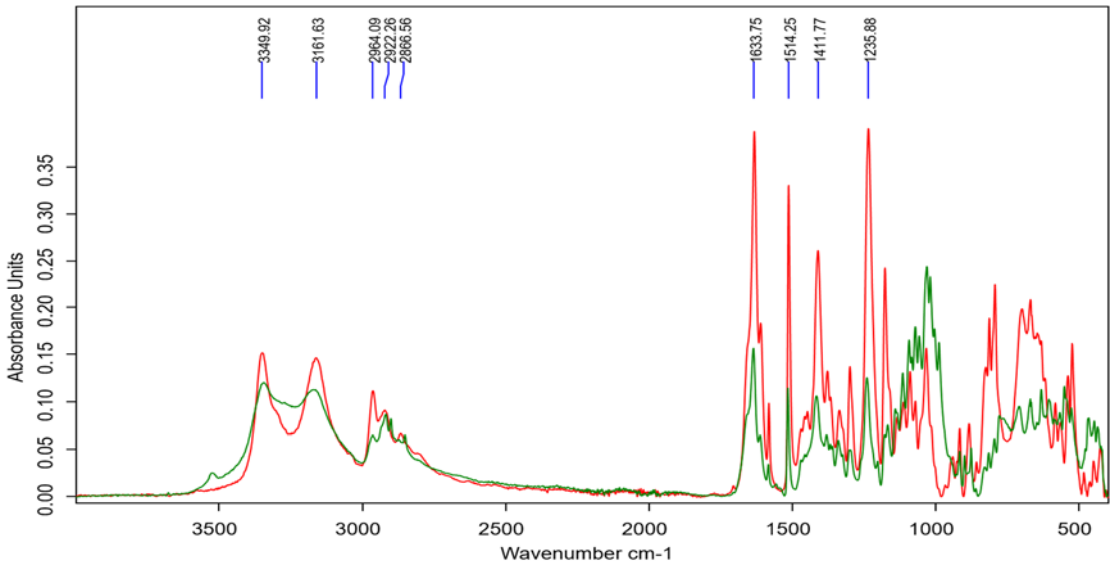

Figure 5.42: ATR FTIR Spectra of At/IND/17 (Green) and atenolol analytical reference (Red)

The spectrum for the At/IND/17 (Figure 5.42) exhibited characteristic peaks for atenolol, but was significantly different to the two characteristic tablet spectra (Figure 5.39 and Figure 5.40) recorded earlier. Differences were observed in the following spectral regions 3500cm^{-1}, 1000cm^{-1} and 800 – 600cm^{-1}. The aromatic peak at 1514cm^{-1} is not masked by any excipient s present in this tablet formulation and is clearly identifiable.

The excipient list for this tablet was unavailable, however using the excipient reference spectra and performing a library search it is possible to infer that lactose, maize starch, silicon dioxide and MCC are present in the tablet. Further work is needed to confirm this.

Overall none of the excipients used in the At/IND/17 tablet formulation exhibit a peak at 1514cm^{-1} and a clear sharp peak can be observed and is not masked or peak shape altered by other excipients, unlike other characteristic atenolol peaks. Whilst we can still detect the hydroxyl, amine and aliphatic CH peaks, the peak shape is slightly different and a range of excipients exhibit peaks in these regions. Thus, enabling the aromatic ring peak to be used as a marker to detect atenolol in the tablet and could be used to quantify the amount of atenolol in it.

5.1.3.1.1.3 Pakistan Atenolol Tablets

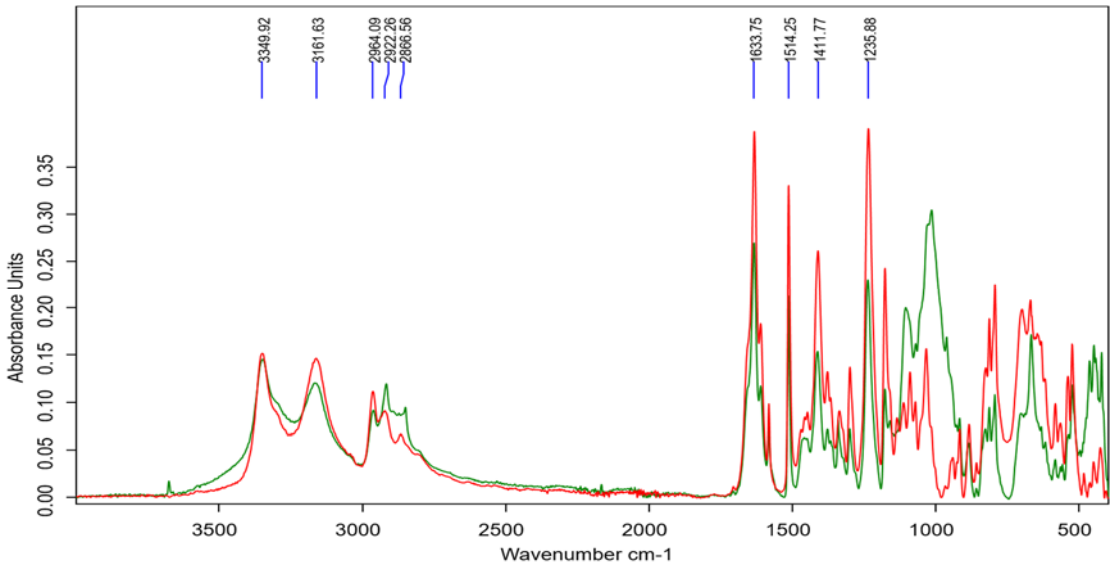

Figure 5.43: ATR FTIR Spectra of At/PAK/1 (Green) and atenolol analytical reference (Red)

The spectra for the At/PAK/1 50mg tablet (Figure 5.43) exhibited characteristic peaks for atenolol. The aromatic peak at 1514cm^{-1} and amide peak at 1236cm^{-1} are not masked by any excipients present in the tablet formulation and are clearly identifiable.

The excipient list for this tablet was unavailable, however using the excipient reference spectra and performing a library search, it is possible to infer that silicon dioxide and starch are present in the tablet. Further work is needed to confirm this.

Overall none of the excipients used in the At/PAK/1 tablet formulation exhibit peaks at 1514 cm^{-1} and 1236cm^{-1}; clear sharp peaks can be observed and are not masked or peak shape altered by other excipients, unlike other characteristic atenolol peaks. Whilst we can still detect the hydroxyl, amine and aliphatic CH peaks, the peak shape is slightly different and a range of excipients exhibit peaks in these regions. Thus, enabling the aromatic ring and amide peaks to be used as markers to detect atenolol in the tablet and could be used to quantify the amount of atenolol in it.

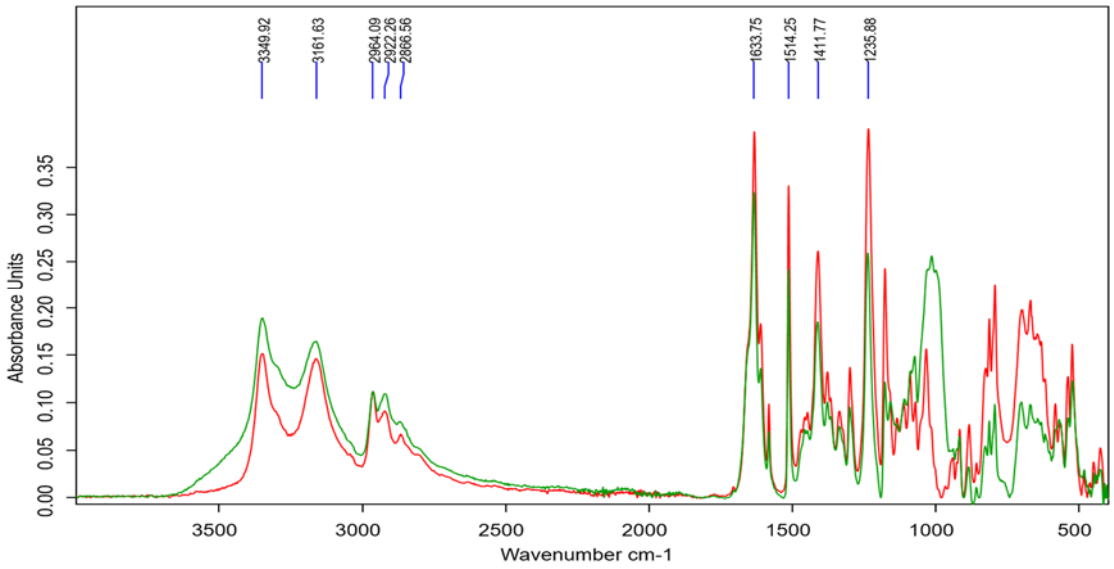

Figure 5.44: ATR FTIR Spectra of At/PAK/2 (Green) and atenolol analytical reference (Red)

The spectra for the At/PAK/2 100mg tablet (Figure 5.44) differs from At/PAK/1 in the spectral region below 1200cm^{-1}.

The excipient list for this tablet was unavailable, however using the excipient reference spectra and performing a library search it is possible to infer that povidone, crosscarmellose sodium, sodium starch glycolate and starch are present in the tablet. Further work is needed to confirm this.

5.1.3.1.1.4 Saudi Arabian Atenolol Tablets

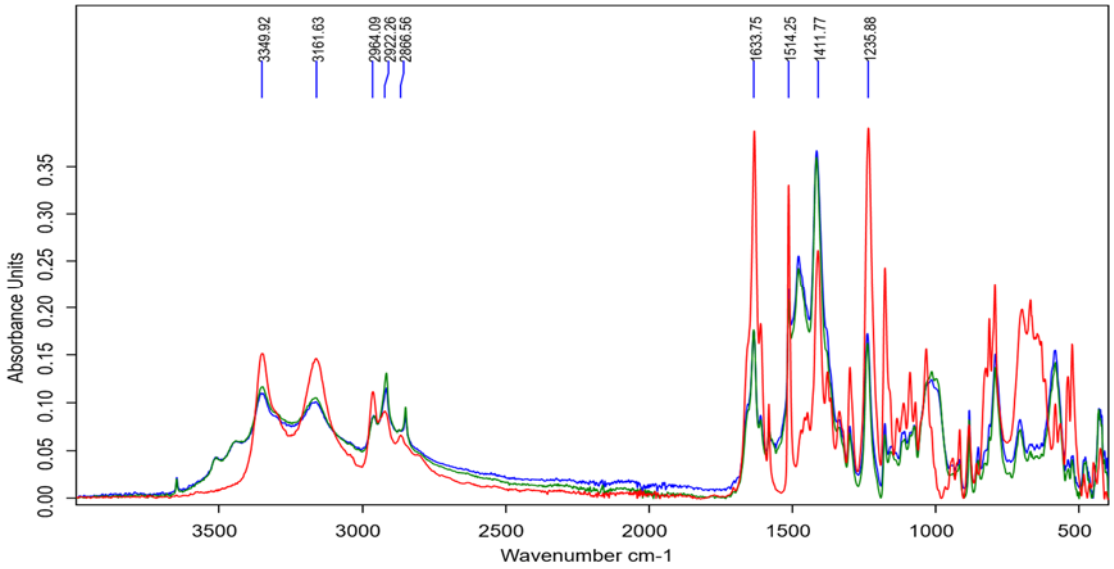

Figure 5.45: ATR FTIR Spectra of At/SA/1 (Green), At/SA/2 (Blue) and atenolol analytical reference (Red)

The spectra for the At/SA/1 and At/SA/2 are identical (Figure 5.45) and exhibited characteristic peaks for atenolol. However, the hydroxyl, amine (3350cm^{-1} and 3152cm^{-1}) and aliphatic CH (2954cm^{-1} and 2922cm^{-1}) and nitro compound (1412cm^{-1}) are not identical to those of the atenolol analytical reference. The aromatic ring peak at 1514cm^{-1} is masked by magnesium carbonate, all other excipients in this tablet are absent at this wavenumber. The amide peak at 1236cm^{-1} can be observed as a clear sharp peak, enabling this to be used as a marker for atenolol and used to quantify the API.

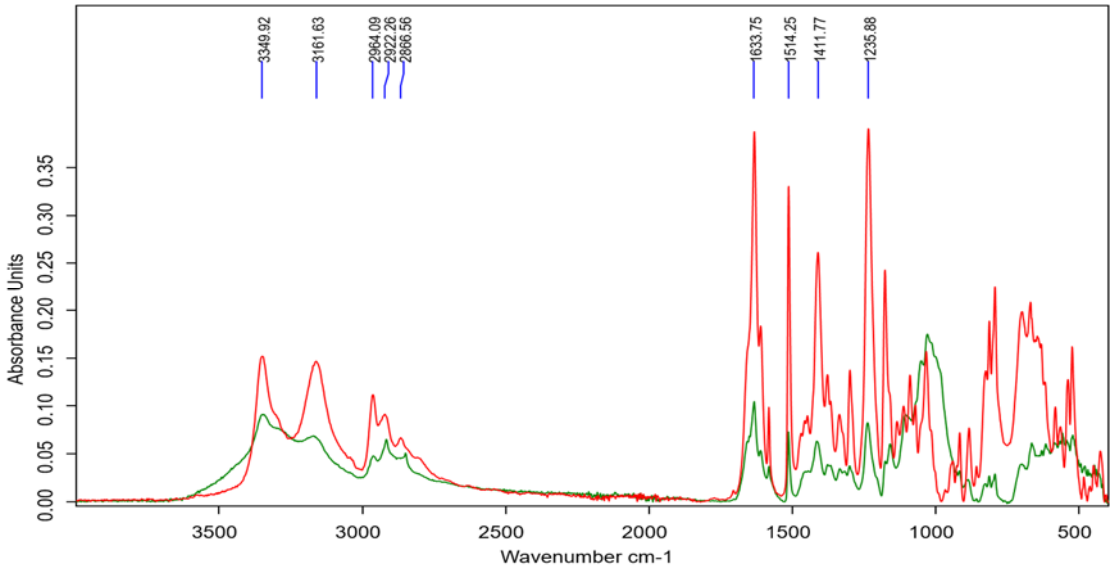

Figure 5.46: ATR FTIR Spectra of At/SA/3 (Green) and atenolol analytical reference (Red)

The spectra for the At/SA/3 100mg tablet (Figure 5.46) exhibited characteristic peaks for atenolol. From looking at the spectra, excipients can be identified within the sample using the excipient reference spectra obtained. Maize starch exhibits a hump in the range of 3500 – 2500cm^{-1}, which could account for the hydroxyl and amine peaks being raised in the spectra. MCC can be identified from the spectra, with peaks exhibited at 1150 – 1000cm^{-1}. Comparing this spectra with other tablets from the same manufacturer (At/SA/4 and At/SA/5), the spectra are identical and the same points can be inferred.

Overall, none of the excipients used in the At/SA/3, At/SA/4 and At/SA/5 tablet formulations exhibit peaks at 1514cm^{-1} and 1236cm^{-1} and clear sharp peaks can be observed and are not masked or peak shape altered by other excipients, unlike other characteristic atenolol peaks.

5.1.3.1.1.5 Nepalese Atenolol Tablets

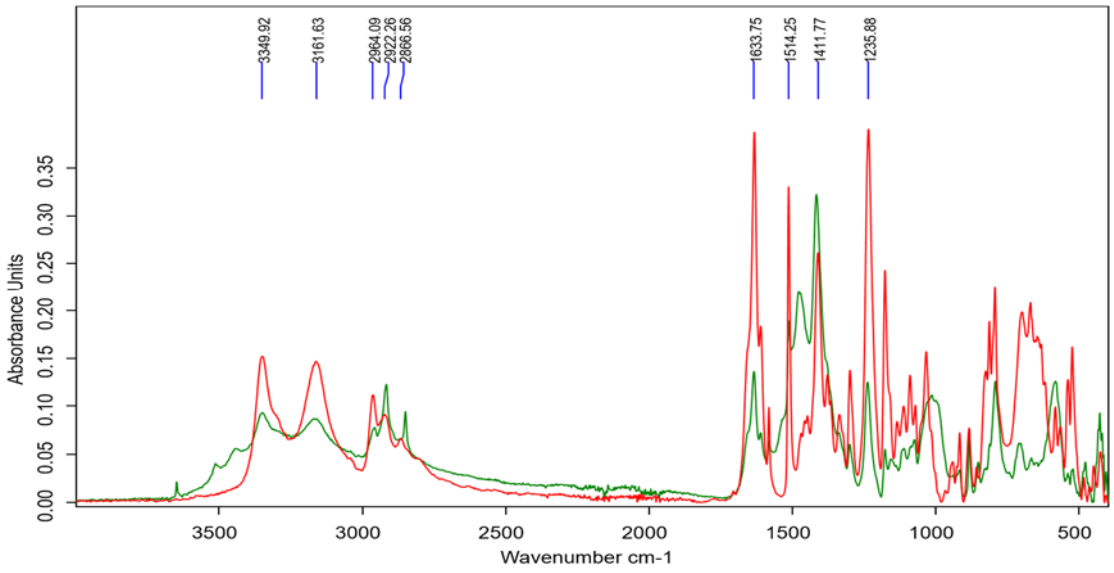

Figure 5.47: ATR FTIR Spectra of At/NEP/1 (Green) and atenolol analytical reference (Red)

The spectrum for the At/NEP/1 sample (Figure 5.47) exhibited characteristic peaks for atenolol. The aromatic ring peak at 1514cm^{-1} is masked by magnesium carbonate, all other excipients in this tablet are absent at this wavenumber. The amide peak at 1236cm^{-1} can be observed as a clear sharp peak, enabling this to be used as a marker for atenolol.

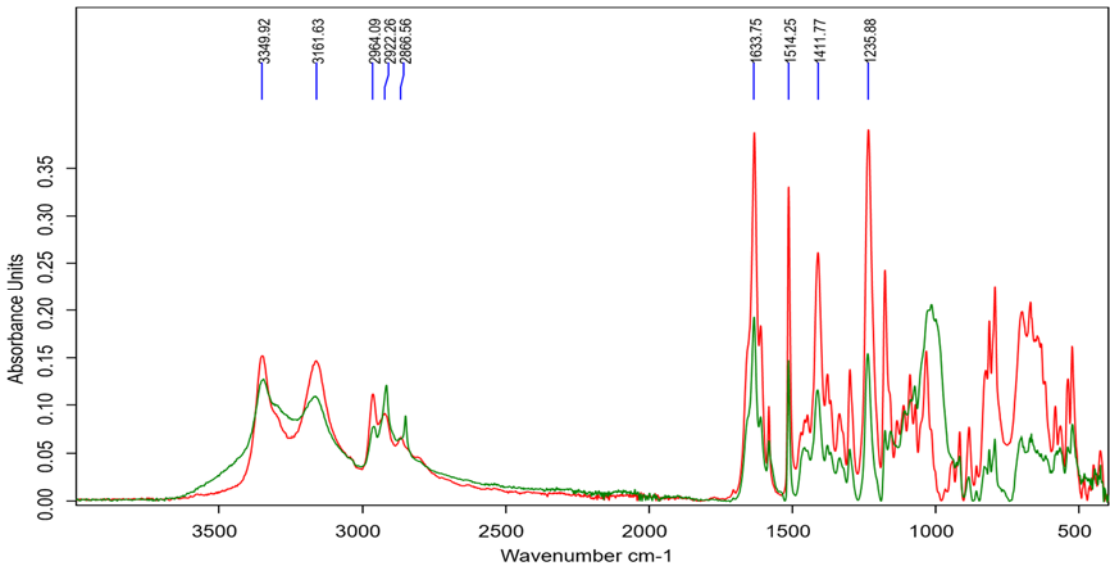

Figure 5.48: ATR FTIR Spectra of At/NEP/2 (Green) and atenolol analytical reference (Red)

The spectrum for AT/NEP/2 50mg tablets (Figure 5.48) exhibited characteristic peaks for atenolol. The aromatic peak at 1514cm^{-1} and amide peak at 1236cm^{-1} are not masked by any

excipients present in the tablet formulation and can be clearly identified. The spectra for the two samples (At/NEP/2 and At/NEP/3) are identical and are the same observations can be made.

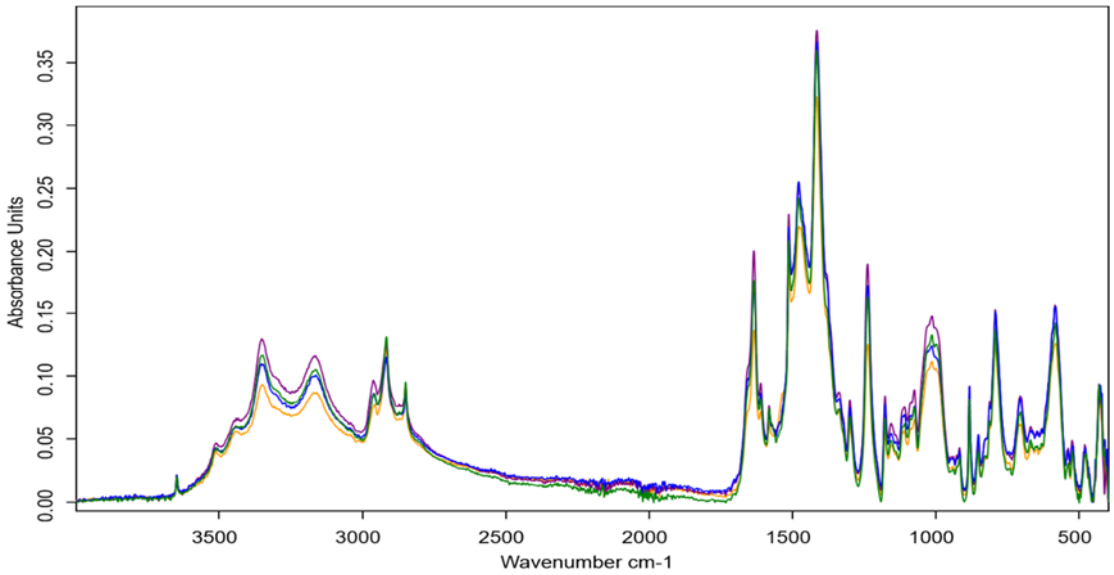

Figure 5.49: ATR FTIR Spectra of At/SA/1 (Green), At/SA/2 (Blue), At/UK/7 (Purple) and At/NEP/1 (Yellow)

The spectrum for At/NEP/1 is very similar to At/UK/7; At/SA/1 and At/SA/2 (Figure 5.49), the formulation of these four tablets should be identical as they are from the same manufacturer. The patient information leaflet for the UK sample indicates the use of the following excipients: gelatin, magnesium carbonate, magnesium stearate, hypromellose, SLS, maize starch and titanium dioxide. The carbonate peak at ~1393cm^{-1} can be observed, as this masks the aromatic peak for atenolol at 1514cm^{-1}.

5.1.3.1.2 UK Metformin Hydrochloride Tablets

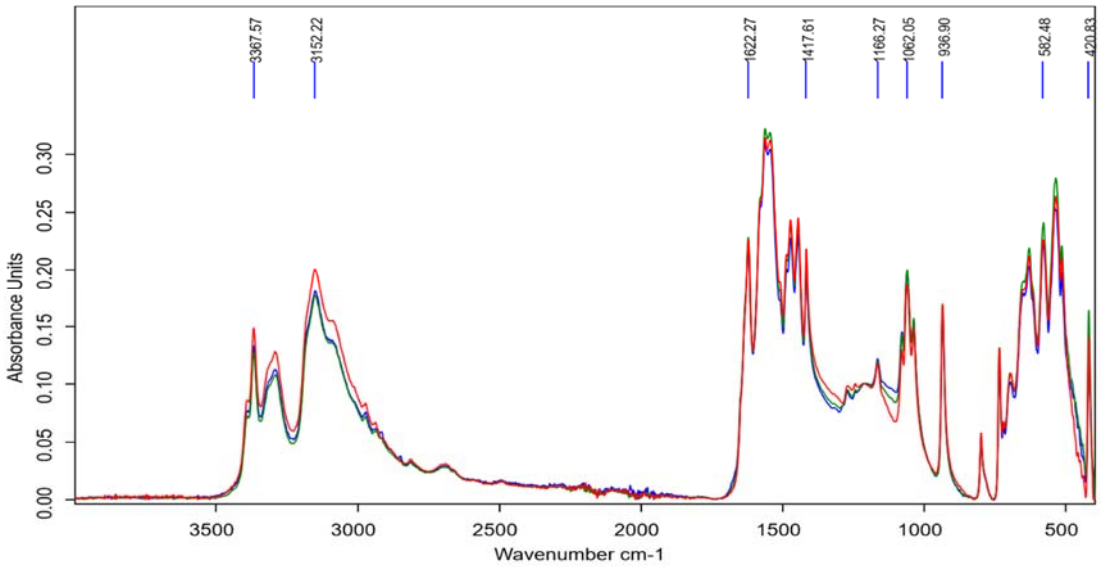

Figure 5.50: ATR FTIR Spectra of Met/UK/1 (Green), Met/UK/2 (Blue) and metformin hydrochloride analytical reference (Red)

The ATR FTIR spectra for the Met/UK/1 and Met/UK/2 tablets are identical (Figure 5.50); therefore, the same observations can be made. The samples exhibited characteristic peaks for metformin hydrochloride at 3368cm^{-1}, 3152cm^{-1}, 1622cm^{-1}, 1418cm^{-1}, 1166cm^{-1}, 1062cm^{-1}, 582cm^{-1} and 421cm^{-1}.

The spectra for the Met/UK/1 and Met/UK/2 tablets matches that of the metformin hydrochloride analytical reference, suggesting that any excipients in the tablet are present in a low concentration and/ or masked by the intense metformin hydrochloride peaks.

5.1.3.1.2.1 Indian Metformin Hydrochloride Tablets

The spectra for the Met/IND/1, Met/IND/2 and Met/IND/3 tablets are identical to the spectra for the UK tablets (Figure 5.50).

Although the samples Met/IND/1, Met/IND/2 and Met/IND/3 are of different dosages, the spectra were identical suggesting that different doses are achieved using tablet of different sizes but of fixed formulation.

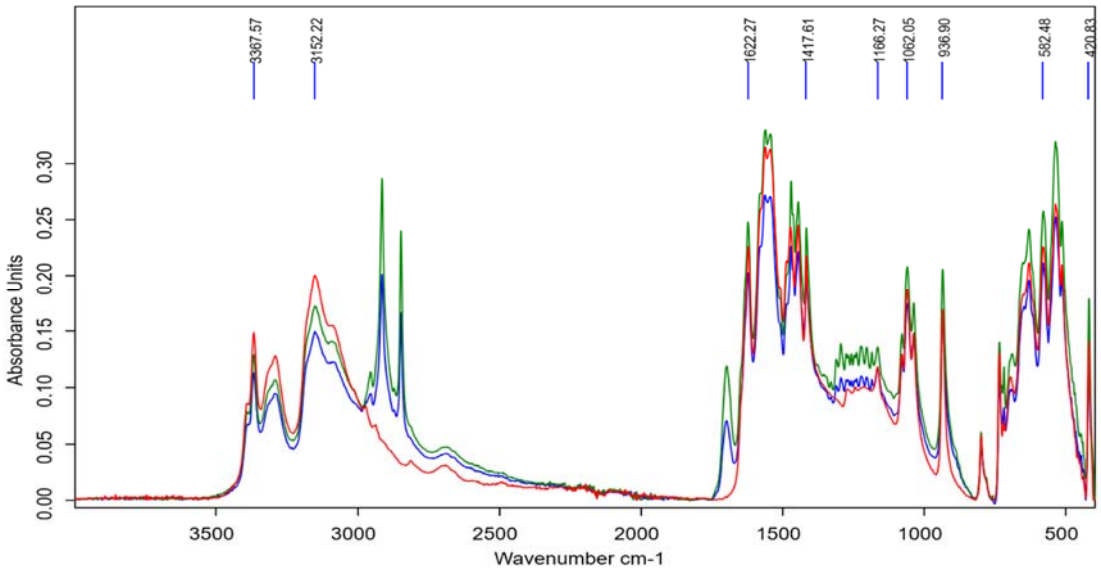

Figure 5.51: ATR FTIR Spectra of Met/IND/4 (Green), Met/IND/5 (Blue) and metformin hydrochloride analytical reference (Red)

The spectra for the Met/IND/4 and Met/IND/5 tablets extended/sustained release samples are identical (Figure 5.51); therefore, the same observations can be made. The samples exhibited characteristic peaks for metformin hydrochloride.

The spectra for the Met/IND/4 and Met/IND/5 tablets resembles that of the metformin hydrochloride analytical reference, however there is the addition of the CH aliphatic peaks (2915cm^{-1} and 2848cm^{-1}) and another peak at 1698cm^{-1}, this could due to the metformin hydrochloride tablet being formulated differently for sustained release. The CH aliphatic peaks are characteristic of metformin hydrochloride (Roselet and Premakumari, 2015), however these peaks may be excipient related, and further work is needed to confirm this. There is also the absence of a CN amine peak at ~1166cm^{-1}.

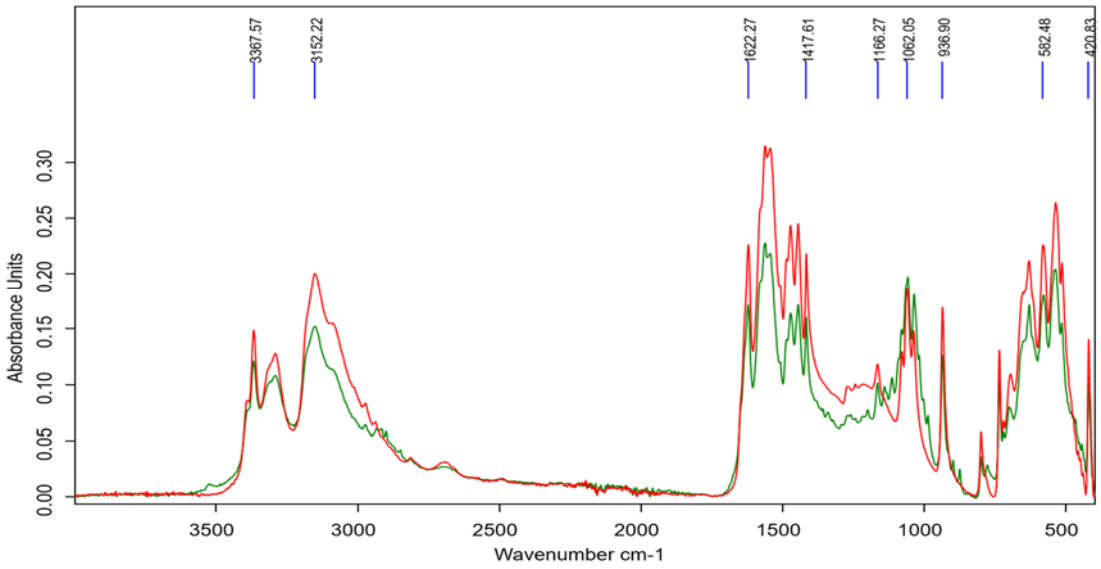

Figure 5.52: ATR FTIR Spectra of Met/IND/6 (Green) and metformin hydrochloride analytical reference (Red)

The spectrum for the Met/IND/6 (Figure 5.52) exhibited characteristic peaks for metformin hydrochloride at 3368cm^{-1}, 3152cm^{-1}, 1622cm^{-1}, 1418cm^{-1}, 1166cm^{-1}, 1062cm^{-1}, 582cm^{-1} and 421cm^{-1}.

The spectrum for the Met/IND/6 tablet matches that of the metformin hydrochloride analytical reference, however, there is a difference at 801cm^{-1}, and this may be due to the addition of another API. Glimepiride is present in a low concentration (1mg) compared to metformin hydrochloride (500mg) and may not be able to be detected, further work is needed to confirm this.

The spectra for the Met/IND/7 and Met/IND/8 were identical to the data shown in Figure 5.50. This demonstrates the comparability of generic formulations and the reproducibility of ATR FTIR analysis of powder samples.

5.1.3.1.2.2 Saudi Arabian Metformin Hydrochloride Tablets

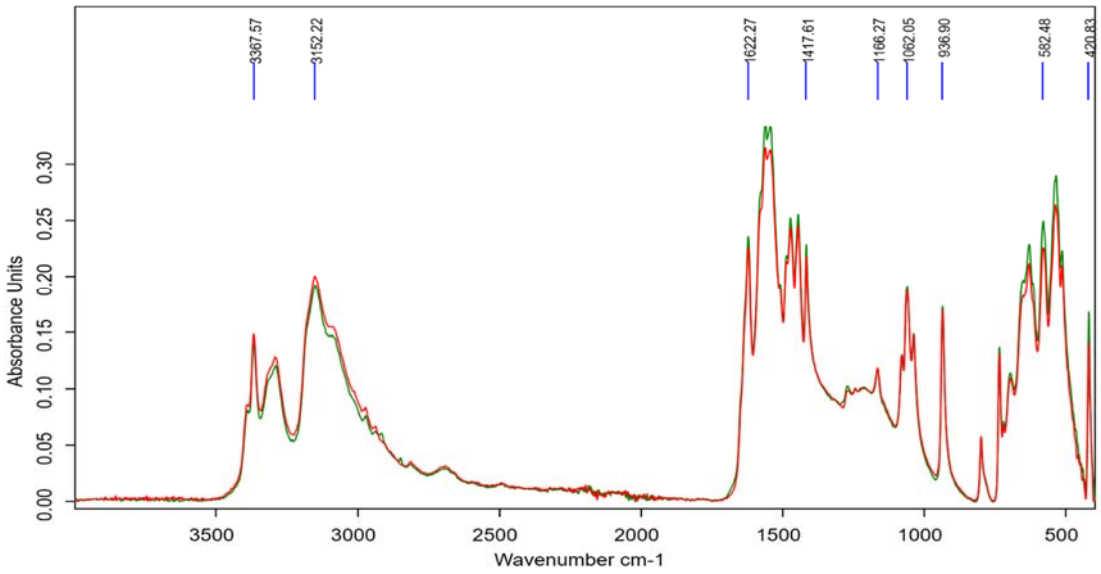

Figure 5.53: ATR FTIR Spectra of Met/SA/1 (Green) and metformin hydrochloride analytical reference (Red)

The spectrum for the Met/SA/1 (Figure 5.53) is identical to the metformin hydrochloride analytical reference, suggesting that any excipients in the tablet are present in a low concentration and/ or masked by the intense metformin hydrochloride peaks. This situation was replicated for all the metformin hydrochloride samples analysed with the exception of the sustained/extended release formulations, where peaks for an additional excipient were clearly visible.

5.1.3.1.3 Antimalarial Tablets

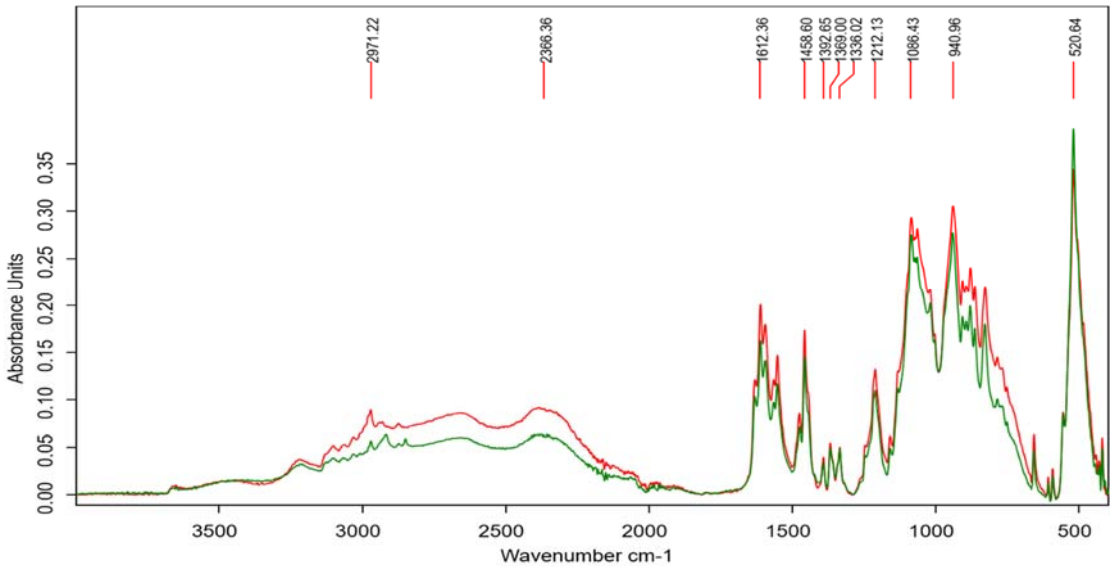

Figure 5.54: ATR FTIR Spectra of AM/UK/1 (Green) and chloroquine phosphate analytical reference (Red)

The spectrum for the AM/UK/1 250mg tablet sample (Figure 5.54) exhibited characteristic peaks for chloroquine phosphate at 3368cm^{-1}, 3152cm^{-1}, 1622cm^{-1}, 1418cm^{-1}, 1166cm^{-1}, 1062cm^{-1}, 582cm^{-1} and 421cm^{-1}. The spectrum for AM/UK/1 is similar to the chloroquine phosphate analytical reference, suggesting that any excipients in the tablet are present in a low concentration and/ or masked by the intense chloroquine phosphate peaks.

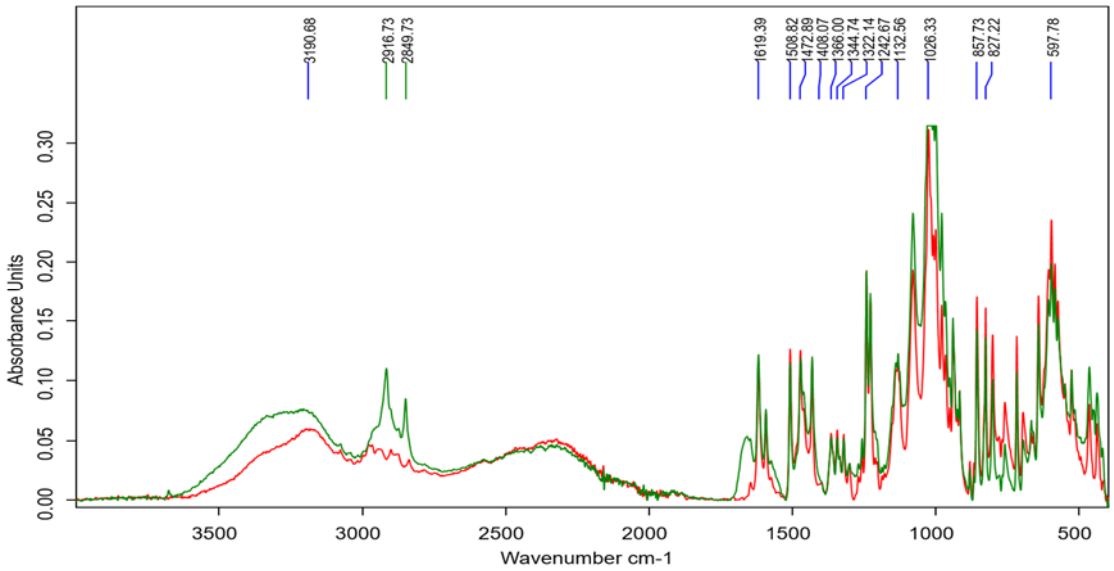

Figure 5.55: ATR FTIR Spectra of AM/ZIM/4 (Green) and quinine sulphate reference (Red)

The spectrum for the AM/ZIM/4 (Figure 5.55) exhibited characteristic peaks for quinine sulphate at 3368cm^{-1}, 3152cm^{-1}, 1622cm^{-1}, 1418cm^{-1}, 1166cm^{-1}, 1062cm^{-1}, 582cm^{-1} and 421cm^{-1}.

The spectrum for AM/ZIM/4 is similar to the quinine sulphate reference; however, there are additional peaks at 3200cm^{-1}, 2917cm^{-1}, 2850cm^{-1} and 1630cm^{-1}, suggesting these may be excipient related. Further work is required to determine this. The peaks at 2917cm^{-1}, 2850cm^{-1} and 1620cm^{-1} are similar to those observed for the powered sustained/extended release metformin hydrochloride samples.

Reference spectra for titanium dioxide showed very broad peaks in the IR centred on 3200cm^{-1} and 820cm^{-1}, with a sharper peak at 1628cm^{-1}. The presence of these peaks in this sample correlates well with the more clearly observed peaks in the Raman spectra for the whole tablet samples for AM/ZIM/4.

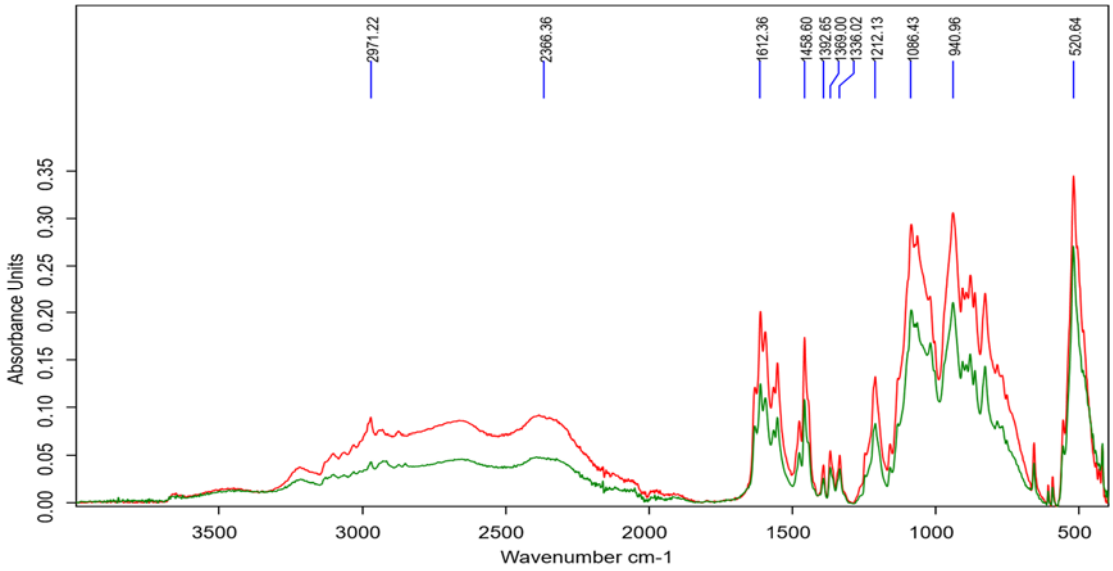

Figure 5.56: ATR FTIR Spectra of AM/NEP/1 (Green) and chloroquine phosphate analytical reference (Red)

The spectrum for the AM/NEP/1 (Figure 5.56) exhibited characteristic peaks for chloroquine phosphate at 2971cm^{-1}, 1612cm^{-1}, 1459cm^{-1} and 1212cm^{-1}. The spectrum for AM/NEP/1 is similar to the chloroquine phosphate analytical reference, suggesting that any excipients in the tablet are present in a low concentration and/ or masked by the intense chloroquine phosphate peaks.

The spectrum for the AM/NIG/3 exhibited characteristic peaks for chloroquine sulphate at 3368cm^{-1}, 3152cm^{-1}, 1622cm^{-1}, 1418cm^{-1}, 1166cm^{-1}, 1062cm^{-1}, 582cm^{-1} and 421cm^{-1}.

5.1.3.2 *Raman Spectroscopy*

5.1.3.2.1 Atenolol

For the majority of tablets there was no significant differences between spectra for the whole tablet and powdered tablet samples. Differences were observed for the colour-coated samples (At/UK/1-6 and At/SA/3-5).

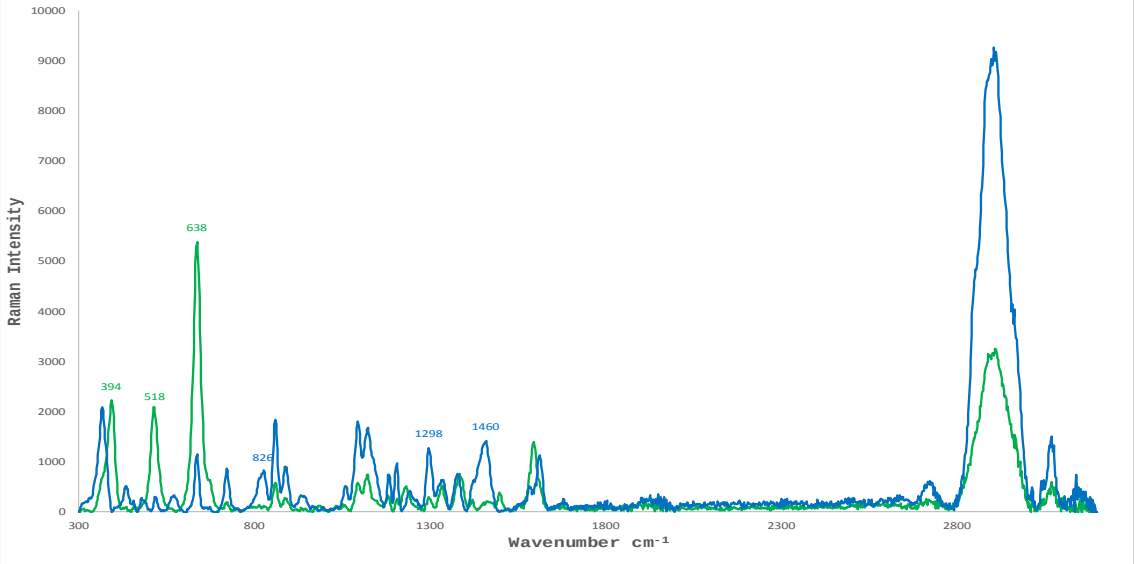

Figure 5.57: Raman Spectra of At/SA/3 whole (Green) and powdered (Blue) tablets using the BRAVO spectrometer

Clear differences between the powdered and whole tablets using the BRAVO spectrometer (Figure 5.57) were observed for the tablet samples for At/SA/3, At/SA/4 and At/SA/5. The spectra for the whole tablet were at a weaker peak intensity and showed some peaks at a slightly different wavenumber and others absent. The peaks at 394cm^{-1}, 518cm^{-1} and 638cm^{-1} for the whole tablet can be attributed to titanium dioxide; this may be due to the coating of the tablet. The peaks at 826cm^{-1}, 1298cm^{-1} and 1460cm^{-1} for the powdered tablet are characteristic of atenolol. There was good agreement for the spectra for At/SA/3, At/SA/4 and At/SA/5, results for At/SA/4 and At/SA/5 are not shown as a result of this.

5.1.3.2.2 Metformin Hydrochloride

The metformin hydrochloride samples analysed using the MIRA-3 handheld spectrometer were powdered into a homogenous sample.

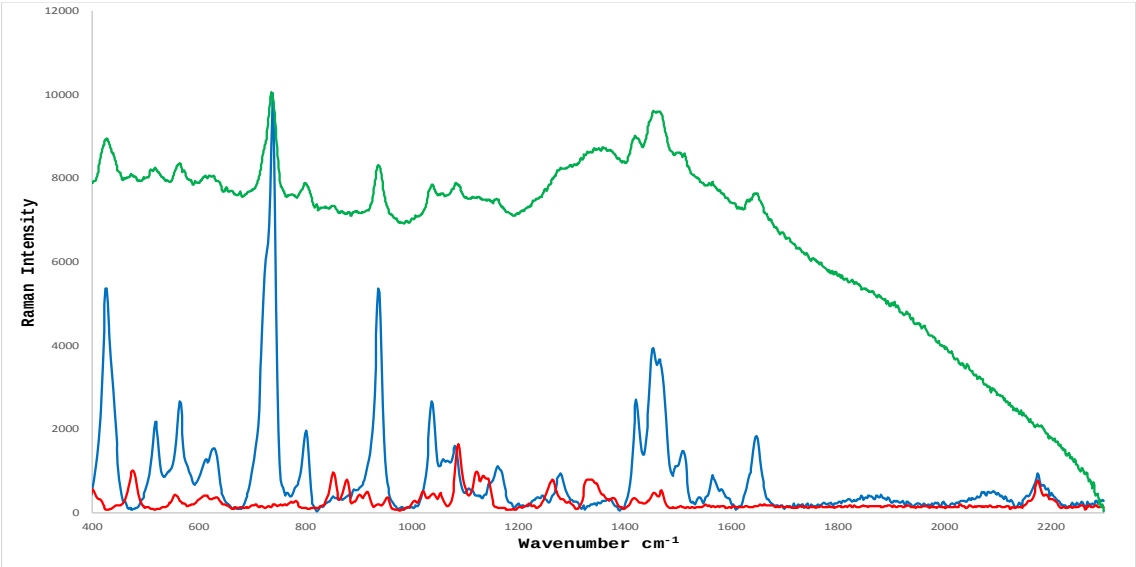

Figure 5.58: Raman Spectra of Met/IND/6 (Blue) and score line (Red) whole tablet using the FORAM-2 and crushed (Green) tablet using the MIRA-3 spectrometer

There were no significant differences between the samples analysed except for Met/IND/6 (Figure 5.58). Differences in the spectra were observed between the benchtop and the handheld systems, with the MIRA-3 spectra less clearly resolved.

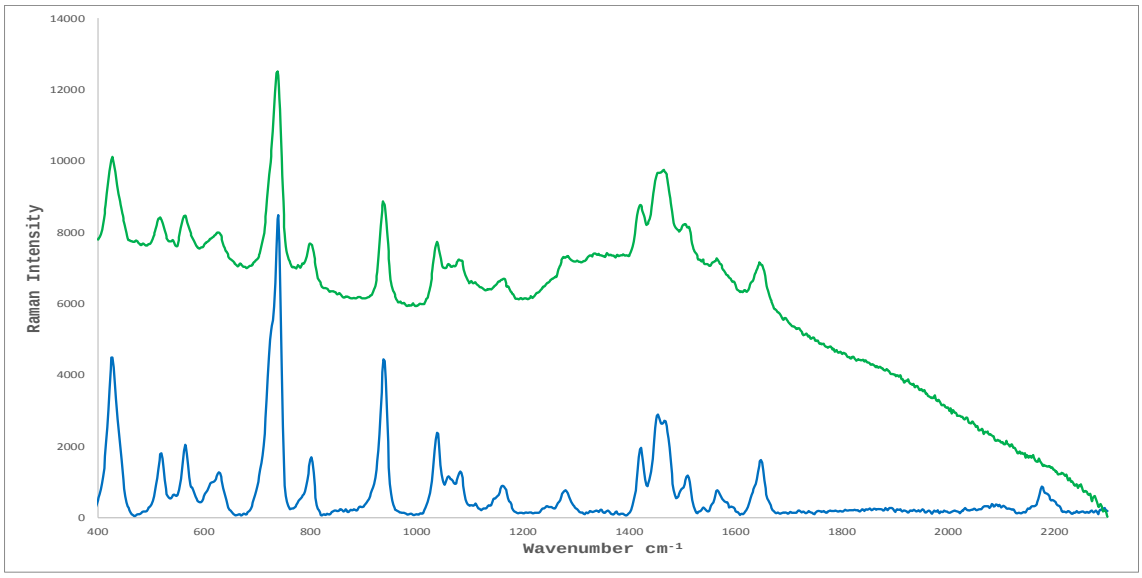

Figure 5.59: Raman Spectra for Met/IND/2 tablets using the MIRA-3 (Green) and FORAM-2 (Blue) spectrometers

However, the spectra of some of the tablets analysed by the MIRA-3 spectrometer showed the API characteristic peaks at a weaker intensity, and appeared to suffer from the effect of fluorescence (Figure 5.59). The same tablets were then analysed whole using the FORAM spectrometer and the peaks for metformin hydrochloride were clearly resolved (Figure 5.59).

5.1.3.2.3 Antimalarials

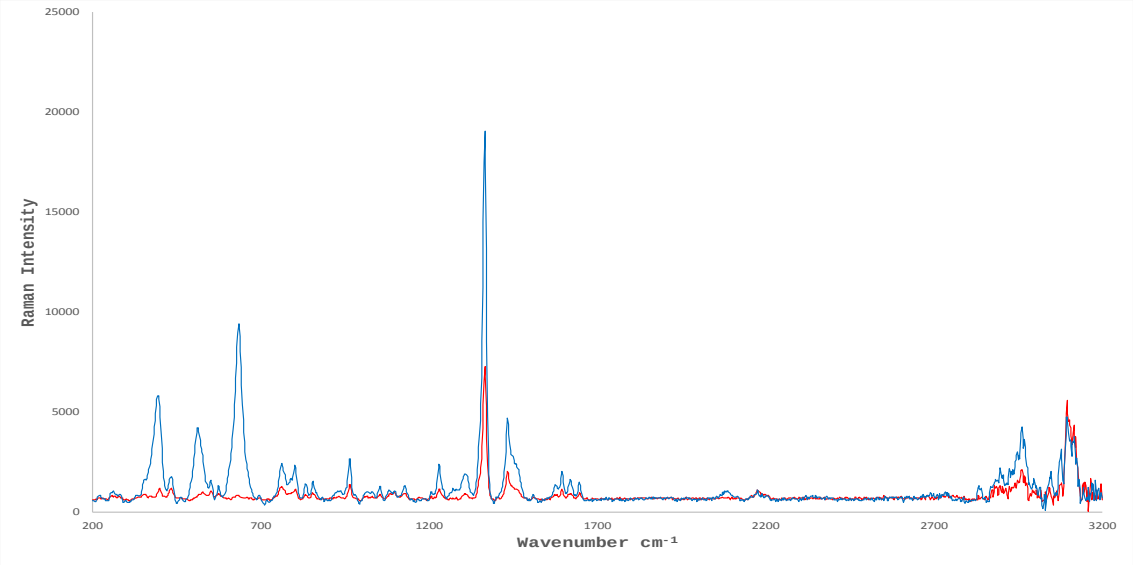

Figure 5.60: Raman Spectra for AM/ZIM/4 whole tablet (Blue) and powdered (Red) using the FORAM-2 spectrometer

Clear differences between the powdered and whole tablets using the FORAM-2 spectrometer (Figure 5.60) were observed for the tablet samples for AM/ZIM/4. The spectra for the whole tablet were at a stronger peak intensity and showed some peaks at a slightly different wavenumber and others absent. The peaks at 394cm^{-1}, 518cm^{-1} and 638cm^{-1} for the whole tablet can be attributed to titanium dioxide; this may be due to the coating of the tablet. The peaks at 1362cm^{-1} and 1430cm^{-1} for the whole and powdered tablets are characteristic of quinine sulphate.

5.1.3.3 *Discussion*

The powdered samples analysed by ATR FTIR gave much more detailed spectra than those for the whole tablets. Significant differences between the two sample types could be observed; well-resolved peaks for the powdered samples enabled the identification of the target APIs when compared to reference spectra. In general, the atenolol ATR FTIR spectra gave similar spectra to one and another, with the exception of excipient interactions. Samples from the same manufacturers showed good agreement and reproducibility of the data. The ATR FTIR spectra for metformin hydrochloride showed good agreement amongst the samples, with the exception of Met/IND/4 and Met/IND/5, the sustained release samples; further work is required to investigate the formulation of these tablets.

Overall, there was no significant differences observed between the whole and powdered tablet sample Raman spectra for atenolol. Differences were identified for colour-coated samples,

suggesting that the sampling penetration depth of Raman did not pass through the coating of these samples. The metformin hydrochloride samples showed no differences in the Raman spectra for whole and powdered tablets, with the exception of Met/IND/6 which contained another API and had two different faces for the whole tablet. The antimalarial sample of AM/ZIM/4 showed differences in the Raman spectra for whole and powdered samples, suggesting that the coating was observed for the whole tablet, as characteristic peaks for titanium dioxide were identified.

5.2 DIP Mass Spectrometry

The samples were analysed in fine powder form as detailed in Chapter 4.3.5.

5.2.1 Reference Spectra

Analytical reference samples for atenolol and quinine sulphate and chloroquine phosphate were analysed using DIP Mass Spectrometry. Total ion chromatogram (TIC) and spectra were recorded and compared. MS data obtained was validated and compared to the National Institute of Science and Technology (NIST) database. (NIST, 2017)

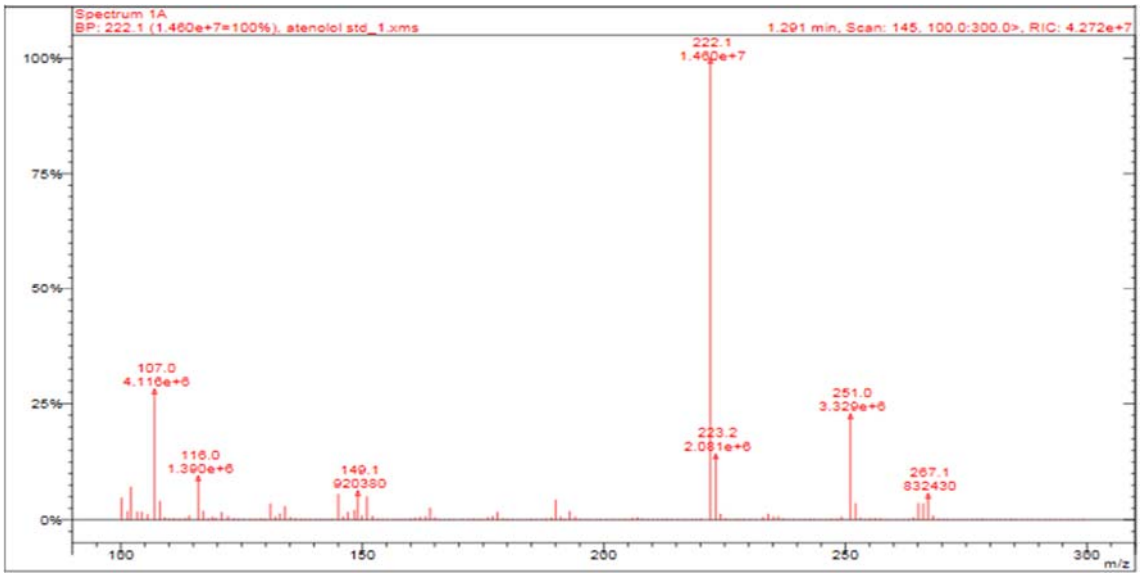

Figure 5.61: DIP MS spectra for atenolol analytical reference

The TIC for atenolol showed a large peak at 65ºC, the mass spectra (Figure 5.61) was then extracted from the TIC data, the presence of ions at m/z 222 (100%), m/z 107 (28%), m/z 251 (23%), m/z 223 (18%), m/z 116 (10%), m/z 149 (8%) and m/z 267 (5%) were detected. The prominent fragment ions at m/z 222, m/z 251 and m/z 223 are characteristic of atenolol, which has a molecular weight of 266g/mol.

On comparison of this work with Carlsen and Aase (1994), who performed similar experiments with deuterated atenolol, identified the production of similar major ions by electron impact DIP process at m/z 223 and m/z 251.

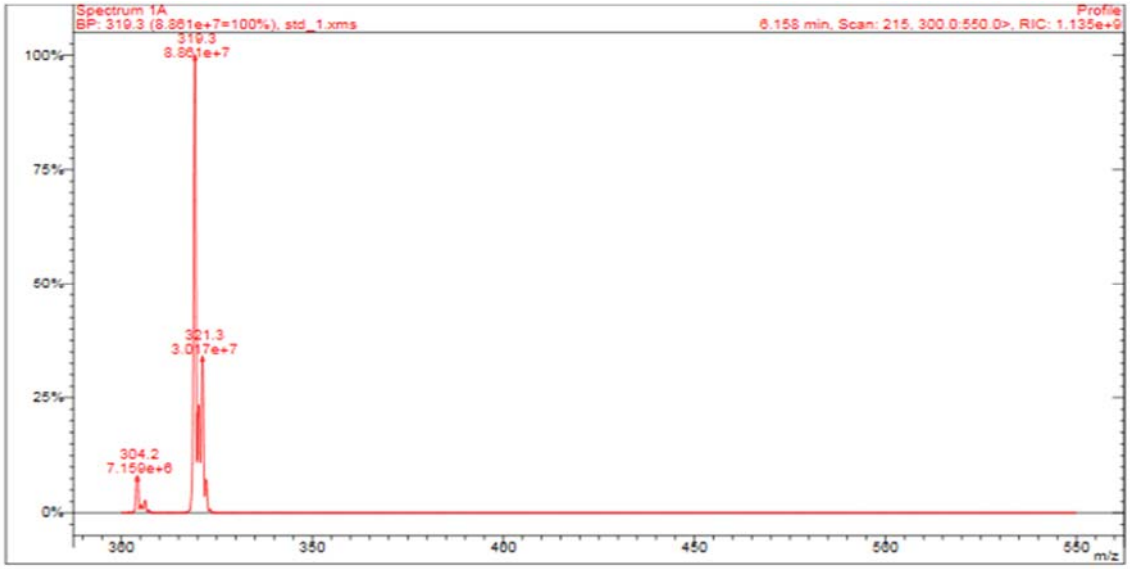

Figure 5.62: DIP MS spectra for chloroquine phosphate analytical reference

The TIC for chloroquine phosphate showed a large peak at 232ºC, the mass spectra (Figure 5.62), was then extracted from the TIC data, the presence of ions at m/z 319 (100%), m/z 321 (30%) and m/z 304 (18%) were detected. The prominent fragment ions at m/z 304, m/z 319 and m/z 321 are characteristic of chloroquine. The NIST database reports that the most intense m/z peak for chloroquine to be m/z 86, some 10 times more intense than the m/z 319 peak. The m/z 319 peak is more characteristic of chloroquine. In this work, the selected mass range excluded the detection of the m/z 86 peak. It should be noted that chloroquine diphosphate has a molecular weight of 516g/mol.

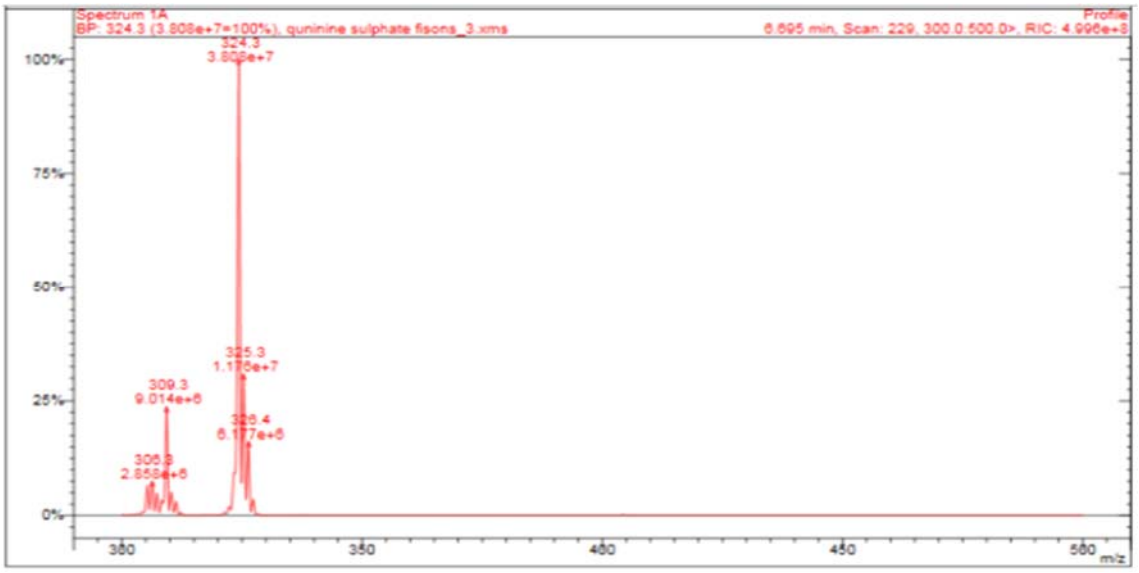

Figure 5.63: DIP MS spectra for quinine sulphate analytical reference

The TIC for quinine sulphate showed a large peak at 130ºC, the mass spectra (Figure 5.63) was then extracted from the TIC and the presence of ions at m/z 324 (100%), m/z 325 (30%), m/z 309 (23%), m/z 326 (15%) and m/z 306 (8%) were detected. The prominent fragment ions at m/z 306, m/z 324 and m/z 325 are characteristic of quinine sulphate. The molecular weight of quinine sulphate is 324g/mol.

5.2.2 Powdered Tablet Sample Studies

5.2.2.1 *Atenolol*

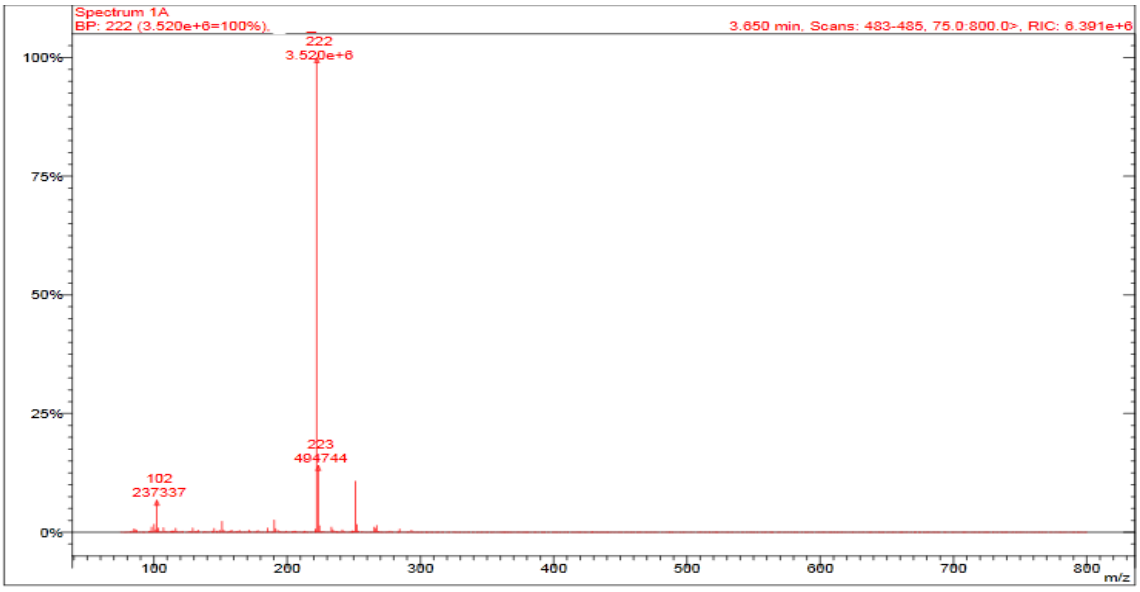

Figure 5.64: DIP MS spectra for At/UK/5

The TIC for At/UK/5 showed a large peak at 80ºC, the mass spectra (Figure 5.64) was then extracted from the TIC data and the presence of ions at m/z 222 (100%), m/z 223 (15%), m/z 251 (13%) and m/z 102 (8%) were observed, which are characteristic of atenolol (Carlsen and Aase, 1994).

This data was characteristic of the UK atenolol samples.

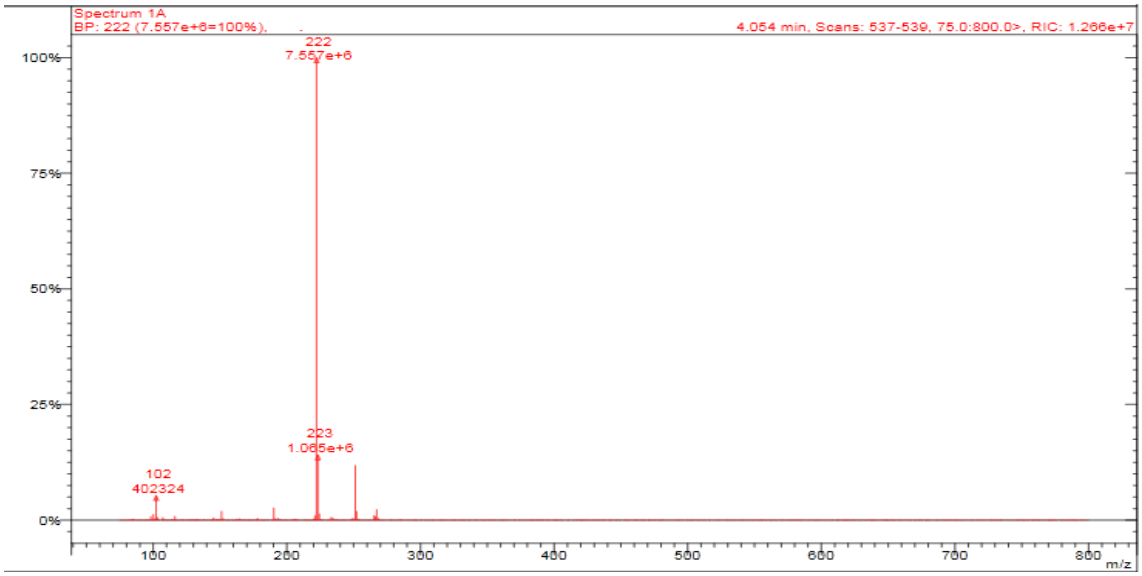

Figure 5.65: DIP MS spectra for At/IND/5

The TIC for At/IND/5 showed a large peak at 150ºC, the mass spectra (Figure 5.65) was then extracted from the TIC data and the presence of ions at m/z 222 (100%), m/z 223 (20%), m/z 251 (15%) and m/z 102 (5%) were observed, which are characteristic of atenolol (Carlsen and Aase, 1994). This data was characteristic of the Indian atenolol samples.

5.2.2.2 *Metformin Hydrochloride*

Due to lack of availability of equipment, no DIP data was obtained for metformin hydrochloride. Literature data for the EI mass spectra of metformin hydrochloride in a non-derivatised form is sparse. The molecule is fragile at elevated temperatures and significant fragmentation would be expected.

5.2.2.3 *Antimalarials*

Samples were taken from various countries in Africa, with a range of different API's anticipated to be present. These were chloroquine phosphate, chloroquine sulphate, quinine sulphate, artemether, lumefantrine, sulphadoxine and pyrimethamine. Reference samples for several of these antimalarials were not available due to the cost and literature data was used to provide reference m/z values.

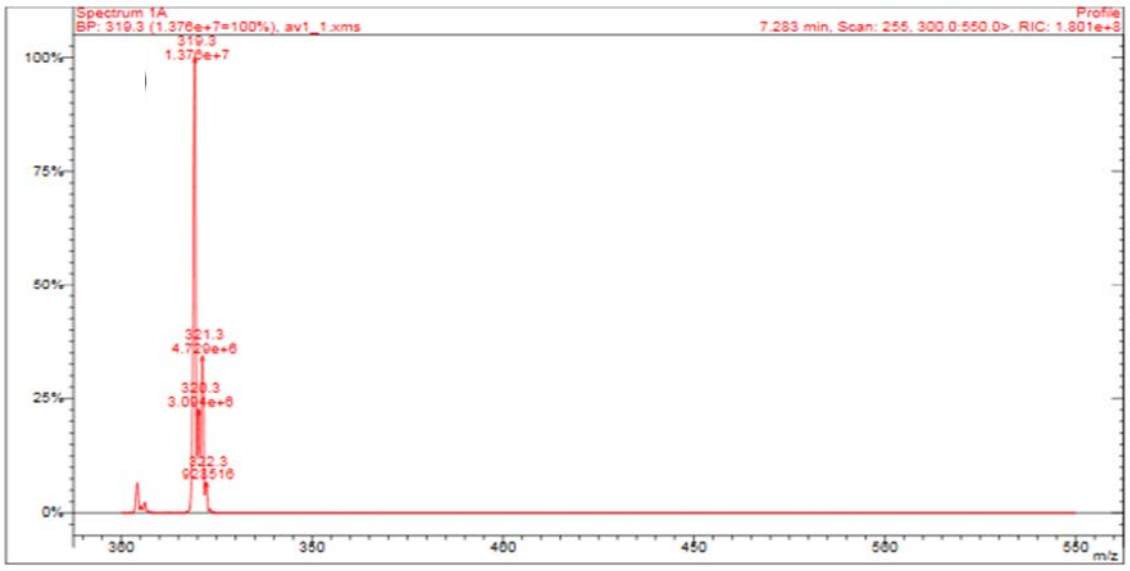

Figure 5.66: DIP MS spectra for AM/UK/1

The TIC for AM/UK/1 showed a large peak at 258ºC, the mass spectra (Figure 5.66) was then extracted from the TIC data and the presence of ions at m/z 319 (100%), m/z 321(33%), m/z 320 (28%), m/z 322 (10%) and m/z 304 (8%) were detected. The prominent fragment ions at m/z 304, m/z 319 and m/z 321 are characteristic of chloroquine phosphate and match those in the chloroquine phosphate analytical standard, confirming the content of the tablet.

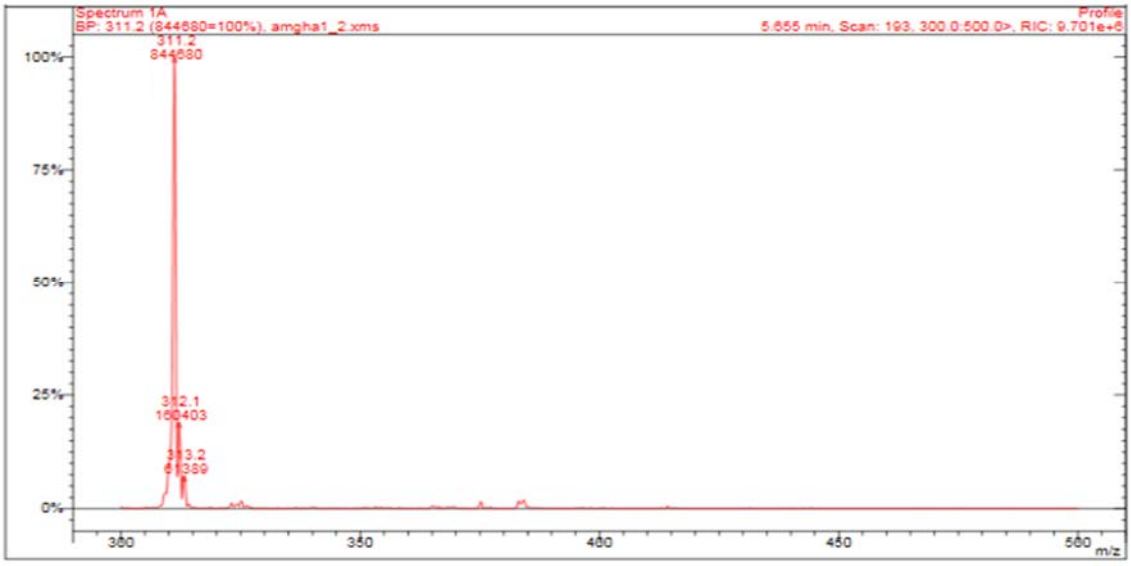

Figure 5.67: DIP MS spectra for AM/GHA/1

The TIC for AM/GHA/1 showed a large peak at 195ºC, the mass spectra (Figure 5.67) was then extracted from the TIC data and the presence of ions at m/z 311 (100%), m/z 312 (18%) and m/z 313 (8%) were detected. This data lacks information on the presence of pyrimethamine m/z 248 and m/z 250 due to the selected scan range m/z 300 – 500. Furthermore, the data for sulphadoxine (m/z 311 and m/z 312) is 1 m/z value higher than expected suggesting poor calibration of the mass range or a high proton affinity for this molecule leading to the formation of the $M + H^+$ species at m/z 311 (Figure 5.76).

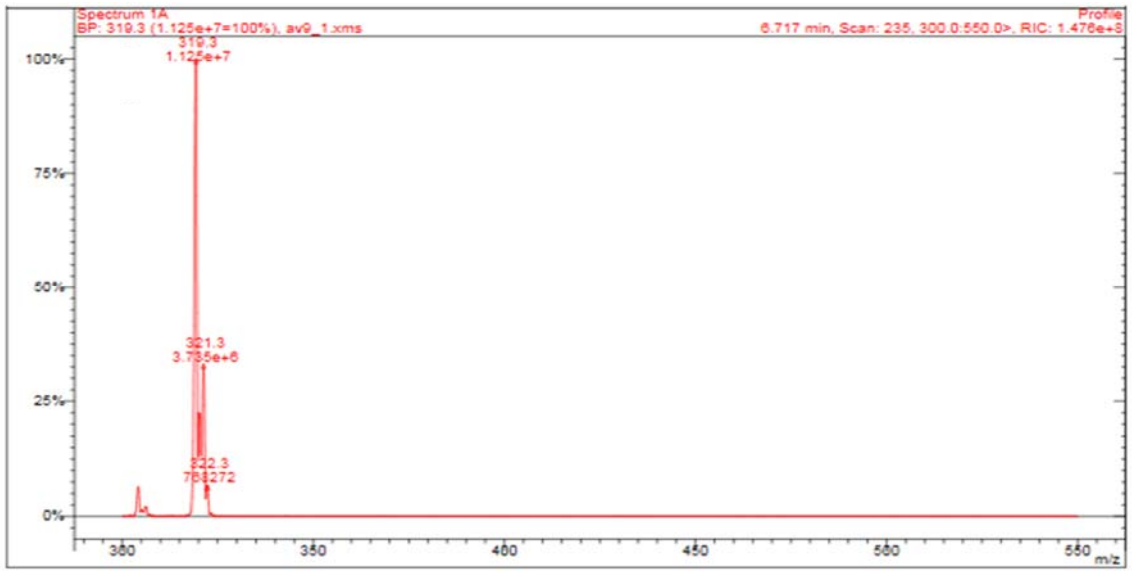

Figure 5.68: DIP MS spectra for AM/NEP/1

The TIC for AM/NEP/1 showed a large peak at 195ºC, the mass spectra (Figure 5.68) was then extracted from the TIC data and the presence of ions at m/z 319 (100%), m/z 321(33%) and m/z

322 (10%) were detected. The prominent fragment ions at m/z 319 and m/z 321 are characteristic of chloroquine phosphate and match those in the chloroquine phosphate analytical standard.

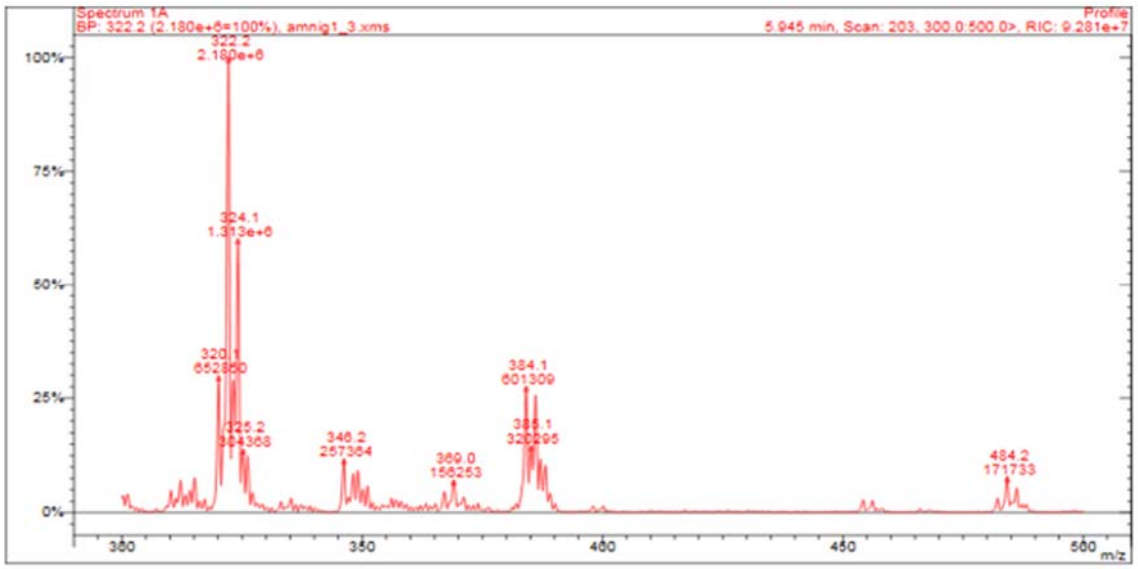

Figure 5.69: DIP MS spectra for AM/NIG/1

The TIC for AM/NIG/1 showed a large peak at 220ºC, the mass spectra (Figure 5.69) was then extracted from the TIC data. The presence of ions at m/z 322 (100%), m/z 324 (60%), m/z 320 (30%), m/z 384 (28%), m/z 385 (15%), m/z 325 (15%), m/z 346 (13%), m/z 484 (8%) and m/z 369 (8%) were detected. The prominent fragment ions at m/z 321, m/z 346, m/z 384 and m/z 484 are characteristic of lumefantrine (NIST 2017).

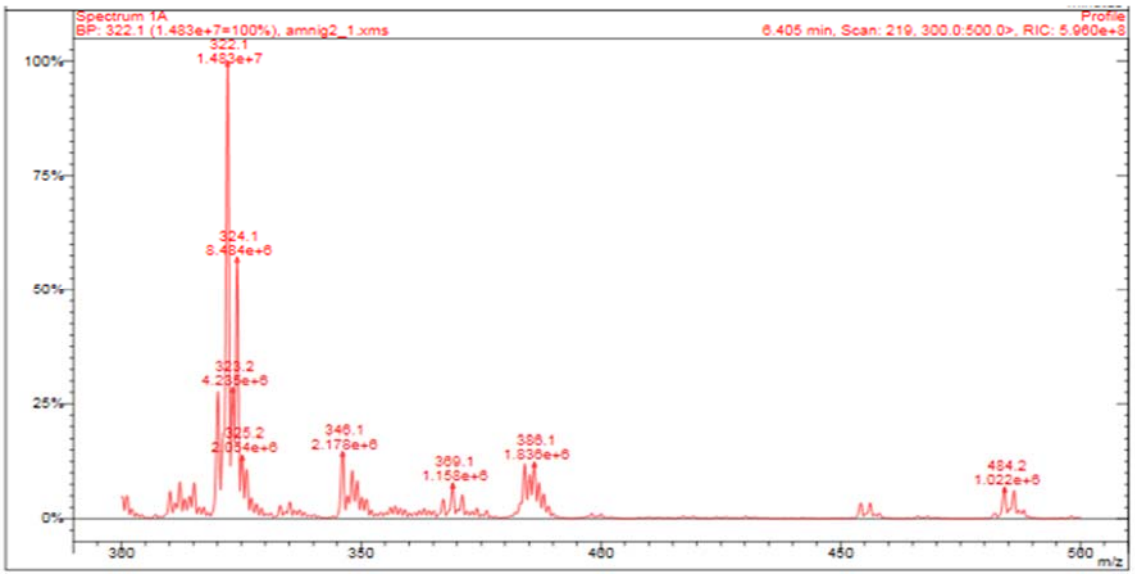

Figure 5.70: DIP MS spectra for AM/NIG/2

The TIC for AM/NIG/2 showed a large peak at 235ºC, the mass spectra (Figure 5.70) was then extracted from the TIC data. The presence of ions at m/z 322 (100%), m/z 324 (28%), m/z 323 (28%), m/z 346 (18%), m/z 325 (15%), m/z 386 (13%), m/z 369 (10%) and m/z 484 (8%) were detected. The prominent fragment ions at m/z 322, m/z 346, m/z 386 and m/z 484 are characteristic of lumefantrine.

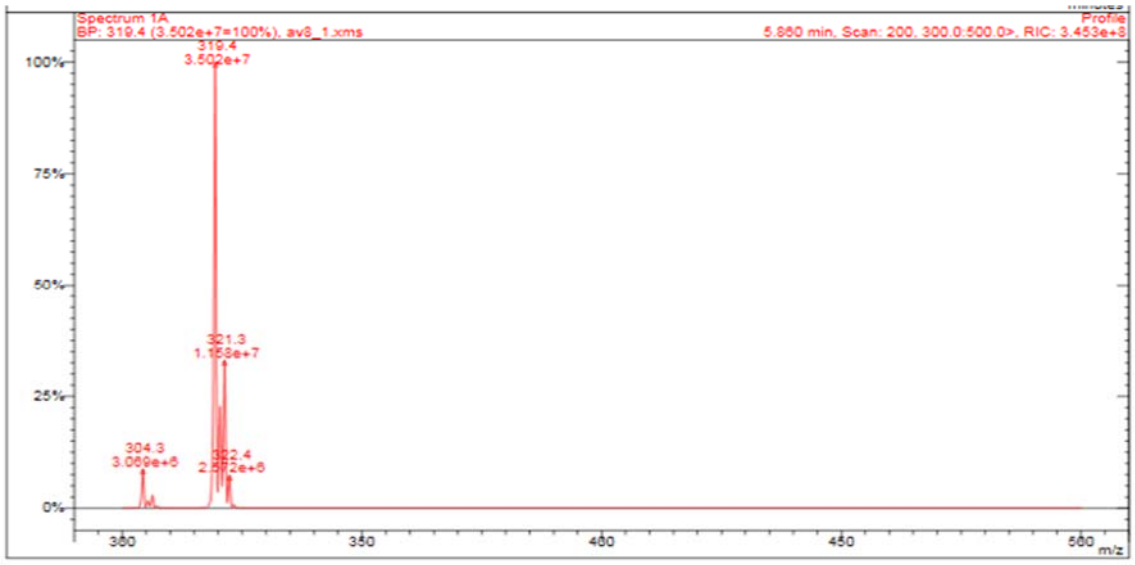

Figure 5.71: DIP MS spectra for AM/NIG/3

The TIC for AM/NIG/3 showed a large peak at 215ºC, the mass spectra (Figure 5.71) was then extracted from the TIC data. The presence of ions at m/z 319 (100%), m/z 321 (33%), m/z 304 (10%) and m/z 322 (8%) were detected. The prominent fragment ions at m/z 304, m/z 319 and m/z 321 are characteristic of chloroquine.

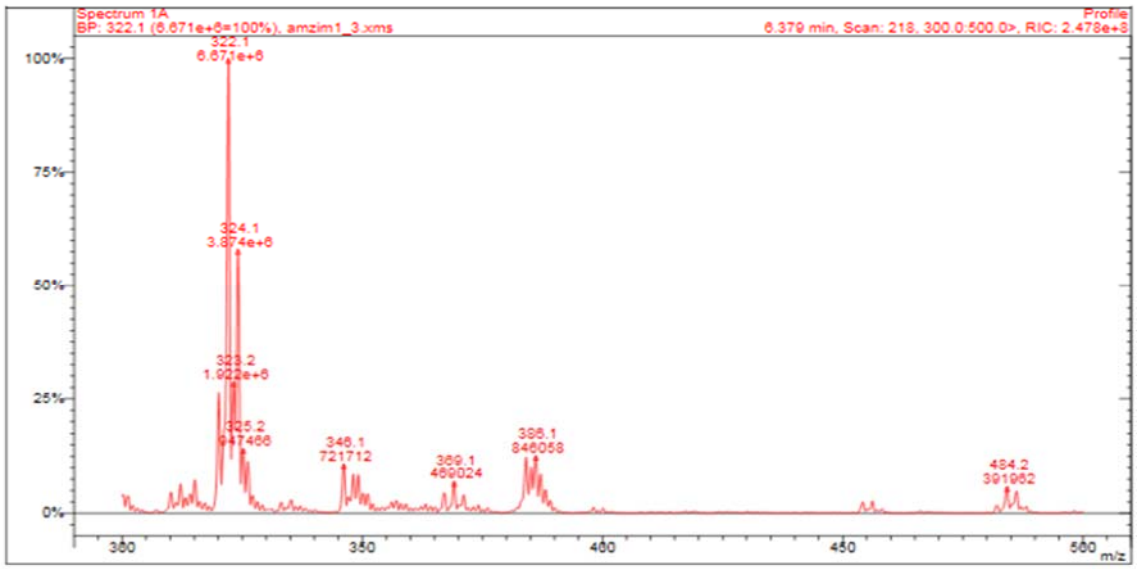

Figure 5.72: Representative DIP MS spectra for AM/ZIM/1 and AM/ZIM/2

The TIC for AM/ZIM/1 exhibited a large peak 230ºC, the mass spectra (Figure 5.72) was then extracted from the TIC data. The presence of ions at m/z 322 (100%), m/z 324 (58%), m/z 323 (28%), m/z 325 (15%), m/z 386 (13%), m/z 346 (10%), m/z 369 (8%) and m/z 484 (5%) were detected. The prominent fragment ions at m/z 322, m/z 346, m/z 386 and m/z 484 are characteristic of lumefantrine (NIST, 2017).

The data for AM/ZIM/2 is comparable to that of AM/ZIM/1 and is not shown.

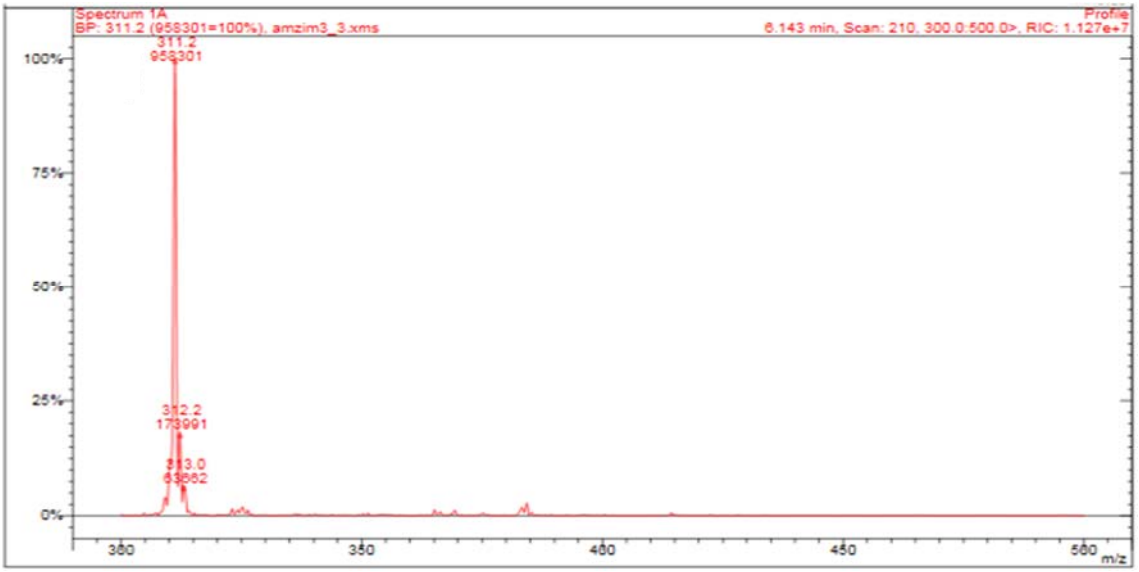

Figure 5.73: DIP MS spectra for AM/ZIM/3

The TIC for AM/ZIM/3 exhibited large peaks at 225ºC, the mass spectra (Figure 5.73) was then extracted from the TIC data and the presence of ions m/z 311 (100%), m/z 312 (20%) and m/z

313 (8%) were detected. This data is identical to that obtained for AM/GHA/1 and the comments made for that sample can be applied here. These tablets merit further investigation (Figure 5.76)

Figure 5.74: DIP MS spectra for AM/ZIM/4

The TIC for AM/ZIM/4 exhibited a large peak at 230ºC, the mass spectra (Figure 5.74) was then extracted from the TIC data and the presence of ions at m/z 324 (100%), m/z 325 (28%), m/z 309 (23%), m/z 323 (8%) and m/z 306 (5%) were detected. The prominent fragment ions at m/z 306, m/z 324 and m/z 325 are characteristic of quinine sulphate.

5.2.3 Discussion

The DIP mass spectra for the tablet samples containing atenolol, chloroquine phosphate and quinine sulphate all confirmed the presence of the API, by reference to the spectra of the analytical standards. The APIs present in the antimalarial samples were sulphadoxine, pyrimethamine, lumefantrine and artemether and no reference samples were available and so a standard scan range was selected. This m/z range was only partially successful as fragmentation led to smaller ions outside of the specified range. However, characteristic data was obtained for these compounds. Further work is needed to repeat these samples for the scan range to include the following values; m/z 221 for artemether, m/z 529 lumefantrine and m/z 153 and 155 for sulphadoxine (M_r 310) and pyrimethamine (M_r 248).

Conventionally new spectrometric identifications are based on the characteristic positive ion fragmentation patterns produced by high-energy electron impact (EI) ionisation processes used in the mass spectrometer (NIST, 2017). This can be summarised as:

$M + e^-_{(H)} = [M^+] + 2e^-_{(L)} = m_1^+ + m_2^+$

Where $e^-_{(H)}$ is high-energy electron and $e^-_{(L)}$ is low energy electron

In many instances, the intensity of the molecular ion (M^+) is significantly reduced. It is also possible for the sample molecule to be ionised by a low energy electron to produce a negatively charged molecular ion, which is unlikely to fragment. The results obtained by this process are compared to data obtained from the new atmospheric pressure or ambient ionisation techniques. In these techniques, the ionisation process can be represented as:

$M + H^+ = (M + H)^+$ (this is the pseudo-molecular ion)

Atmospheric pressure or ambient ionisation techniques offer two technical differences to the EI-MS process. In atmospheric pressure systems ions are created outside the MS vacuum system, usually by the addition of a proton rather than the removal of an electron, and under these conditions the energy transferred to the target molecule is low and little fragmentation is observed. Under these conditions, the target signal is the anticipated molecular weight plus 1 mass unit.

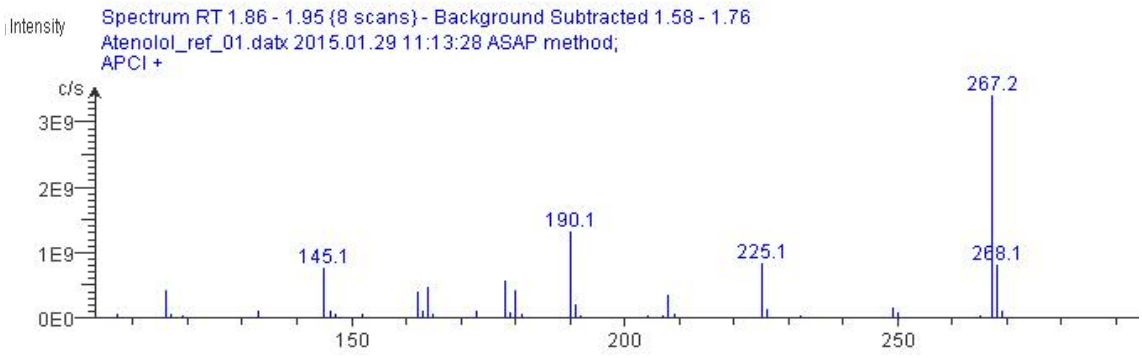

Figure 5.75: ASAP MS spectra for atenolol analytical reference

The ASAP MS spectra shown in Figure 5.75 is much comparable to that obtained for DIP MS, which is surprising because proton transfer is a much softer ionisation than that of EI.

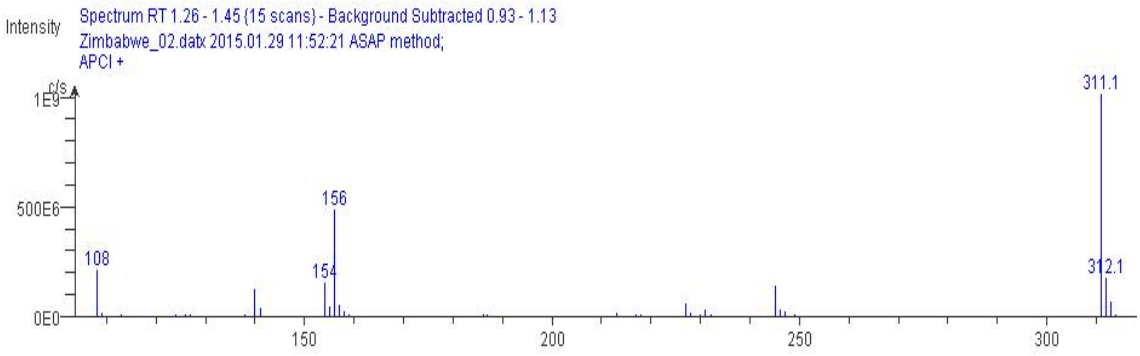

Figure 5.76: ASAP MS spectra for a sulphadoxine/ pyrimethamine tablet from Zimbabwe

Figure 5.76 shows example spectra for antimalarial tablets and again it can be observed that the ASAP spectra shows less fragmentation that the DIP spectra. There is clear evidence of the protonated molecular ion at m/z 311 (for sulphadoxine) and virtually no fragmentation ions. The ions at m/z 248 and 249 are the molecular species for pyrimethamine (M^+ and $M + H^+$). The data for sulphadoxine from this analysis is comparable to the DIP analysis of the same tablets (Figure 5.73) and comparable samples in Figure 5.67. Whilst the data for sulphadoxine was consistent between the samples (all stated to contain this API), the DIP results were unable to confirm the presence of pyrimethamine.

5.3 Summary

The results for the identification of an API within either a whole or crushed tablet were discussed. Raman Spectroscopy was the only analytical method that could identify the API in a whole tablet successfully. The other methods (ATR FTIR, DIP MS and ASAP MS) required the sample to be crushed. There was no significant differences in the wavelengths of the observed absorption bands between the whole tablet and the powdered sample Raman spectra for atenolol and metformin hydrochloride. Tablet samples produced enhanced Raman absorption signals versus the powdered samples. Differences were observed for colour-coated samples, suggesting that the sampling penetration depth of Raman did not pass through the coating. The spectra for the antimalarial sample AM/ZIM/4 showed differences in the Raman spectra for several different whole samples, suggesting that the coating, titanium dioxide, was poorly dispersed on the surface.

The DIP mass spectra for the samples containing atenolol, chloroquine phosphate and quinine sulphate all confirmed the presence of the API. the other antimalarials (sulphadoixine, pyrimethamine, lumefantrine and artemether) analysed were unable to confirm the presence of the API, as the scan range selected was not appropriate and led to few characteristic peaks to be

identified. ASAP spectra showed less fragmentation that the DIP spectra and could be used to identify atenolol, sulphadoxine and pyrimethamine.

Once the target API was identified within a sample, it was possible to quantify the amount of API present. Chapter 6 discusses the quantification of the API within a sample and the results are compared to the standard assay based on data from the British Pharmacopeia.

6 API Quantification and Discussion

This chapter presents and discusses the API quantification results obtained from tablet samples using a selection of analytical techniques as discussed in Chapter 3.

Unlike most analytical projects, the target reference material in this study was the 'innovator tablet'. In the countries under study for this work, generic medicine formulations are common and therefore appropriate FDA and EMA guidelines with respect to generic formulations have been used. These guidelines suggest that generic medicines should contain between 80 – 125% (FDA) (van Gelder, 2017 and Vinks, 2017) or 90 – 110% (EMA) (van Gelder, 2017 and Vinks, 2017) of the dose of the innovator drugs API.

The results presented have been derived from UV, ATR FTIR, Raman and EDX analysis.

6.1 UV Analysis

6.1.1 Atenolol

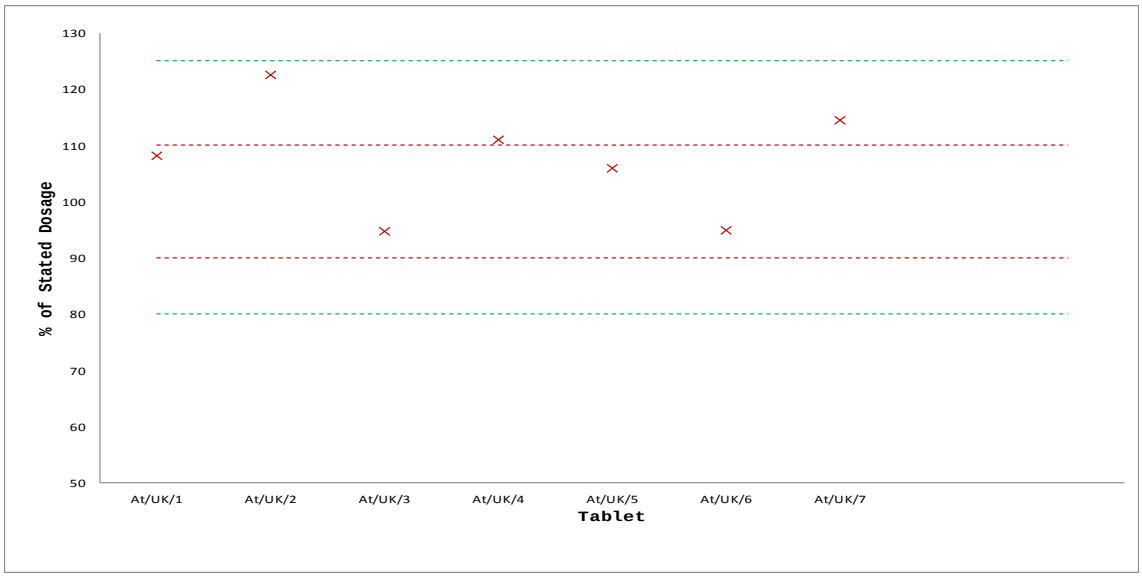

Figure 6.1: Percentage of stated dosage of UK atenolol tablets as calculated by UV with EMA (90-110%) (--) and FDA (80-125%) (--) limits

Figure 6.1 summarises the quantification results for the UK atenolol tablets using UV. The results are mixed with variation between dosage and manufacturer being observed. The At/UK/1, At/UK/3, At/UK/5 and At/UK/6 samples are in the range as outlined by the EMA (90 – 110 %).

The tablets that fell out of this range generally fell above this threshold. The At/UK/2, At/UK/4 and At/UK/7 samples were slightly higher but fell within the FDA range (80 – 125%).

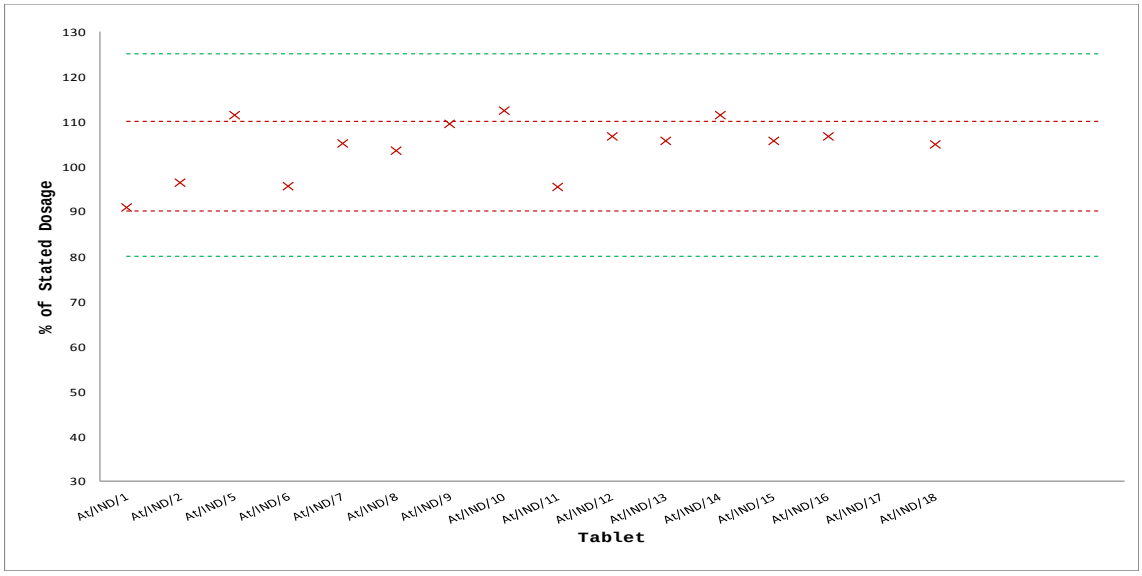

Figure 6.2: Percentage of stated dosage of At/IND/1-18 tablets as calculated by UV with EMA (90-110%) (--) and FDA (80-125%) (--) limits

Figure 6.2 summarises the quantification results for the At/IND/1& 2 and 5 - 18 tablets using UV. The results for Indian atenolol samples all fell within the EMA (90 – 110%) acceptable range, with At/IND/5, At/IND/10 and At/IND/14 just falling short of the acceptable limit, but well within the FDA limits (80 – 125%). Variations in the level of atenolol may be due to batch-to-batch variations.

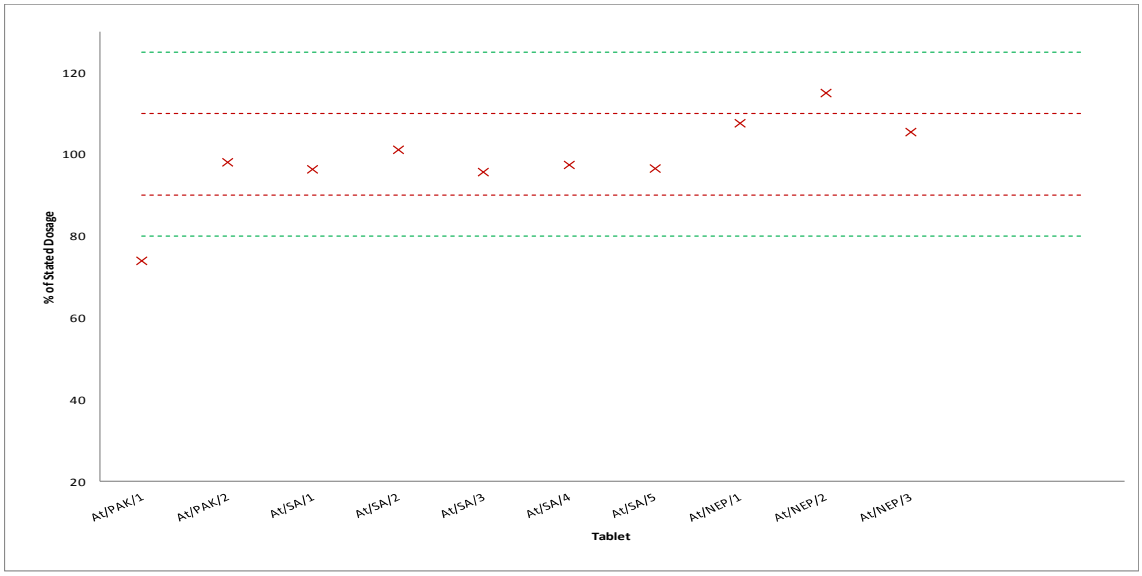

Figure 6.3: Percentage of stated dosage of Pakistani, Saudi Arabian and Nepalese atenolol tablets as calculated by UV EMA (90-110%) (--) and FDA (80-125%) (--) limits

Figure 6.3 summarises the quantification results for the Pakistani, Saudi Arabian and Nepalese atenolol samples. The At/PAK/2 sample fell within the acceptable range, whilst the At/PAK/1 sample was significantly low and considerably outside both the EMA and FDA acceptable limits. This could indicate the possibility of poor quality control or that the sample is counterfeit/ substandard.

The tablets from Saudi Arabia (At/SA/1, At/SA/2, At/SA/3, At/SA/4 and At/SA/5), all fell within the acceptable range for atenolol as outlined by the BP 2017. The tablets from Nepal (At/NEP/1 and AT/NEP/3), all fell within the EMA acceptable range for atenolol except for At/NEP/2. The At/NEP/2 sample appears to contain a higher concentration (115%) of atenolol than expected, but is still within the FDA limit.

6.1.2 Metformin Hydrochloride

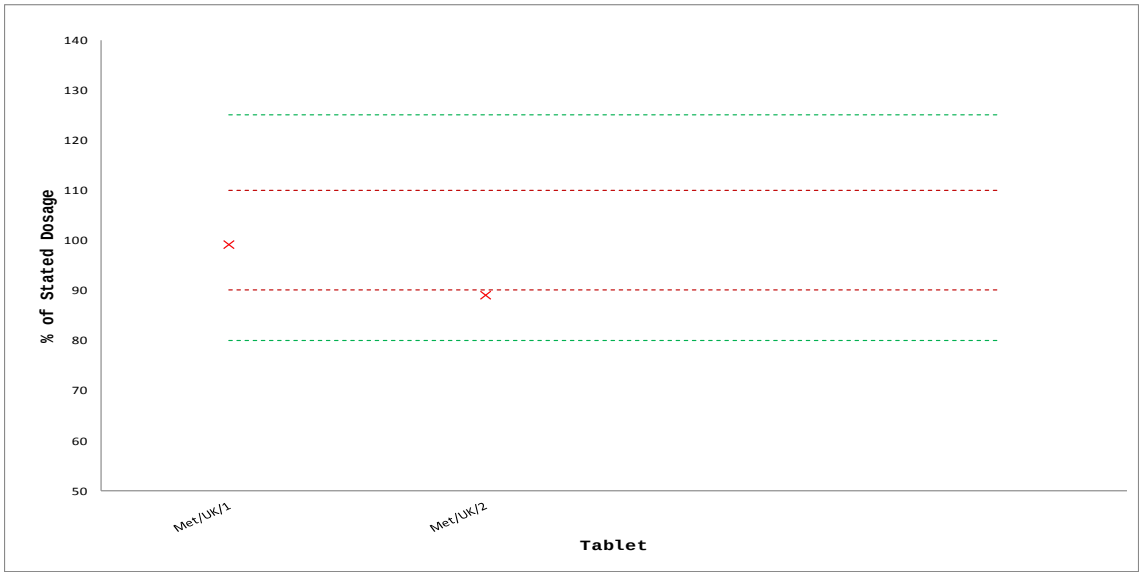

Figure 6.4: Percentage of stated dosage of UK metformin hydrochloride tablets as calculated by UV with EMA (90-110%) (--) and FDA (80-125%) (--) limits

Figure 6.4 summarises the quantification results for the UK metformin hydrochloride tablets using UV. The results are mixed, with variation between dosage and manufacturer being observed. The percentage of stated dose for the Met/UK/2 sample is lower than expected and falls below the acceptable EMA range (90 -110%), but within the FDA limits (80 – 125%).

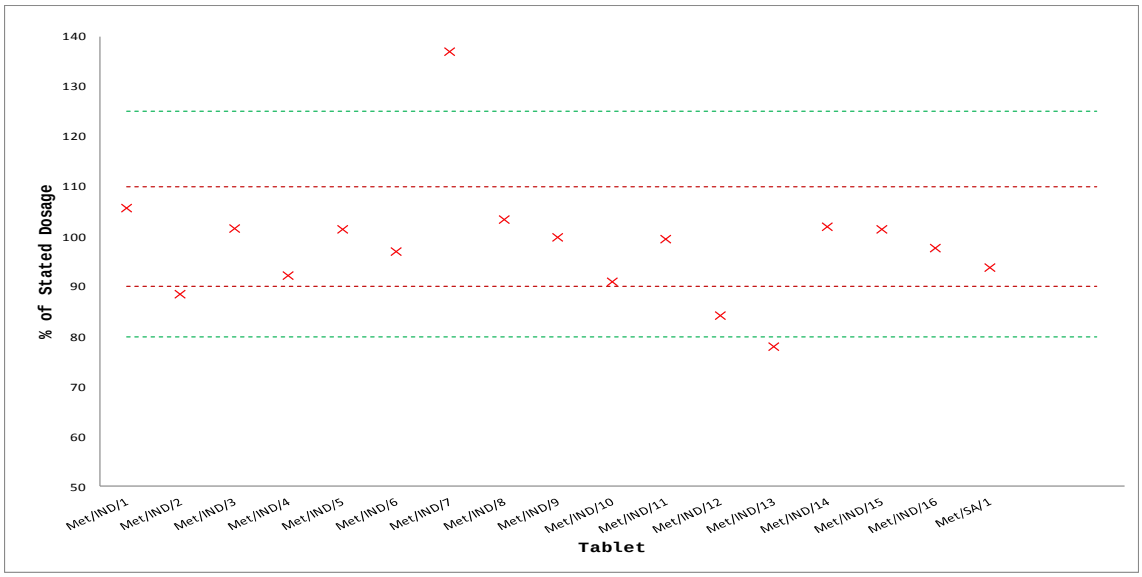

Figure 6.5: Percentage of stated dosage of Indian and Saudi Arabian metformin hydrochloride tablets as calculated by UV with EMA (90-110%) (--) and FDA (80-125%) (--) limits

Figure 6.5 summarises the quantification results for the Indian metformin hydrochloride tablets using UV. Overall, the majority of the tablets fall in the acceptable limits for metformin

hydrochloride. Met/IND/2 and Met/IND/12 are just outside the EMA limits, but within the FDA limits. The Met/IND/13 samples indicate a lower than anticipated level of metformin hydrochloride. The Met/IND/7 is significantly high and considerably outside the both the EMA and FDA acceptable limits. This could indicate the possibility of poor quality control or that the sample is counterfeit/ substandard. This may be related to the overall quality of the tablet, on receipt of the tablets, several of them were broken and appeared to be of poor quality in the blister packaging (Figure 6.6); this may account for the difference in values for the two Met/IND/7 and Met/IND/8 samples, which are from the same manufacturer.

Figure 6.6: Met/IND/7 tablets within the blister packaging

6.2 ATR FTIR Spectroscopy

6.2.1 Atenolol

6.2.1.1 *Calibration mixtures*

Calibration mixtures for the quantification of atenolol in a single tablet were prepared as detailed in Chapter 4.3.1.

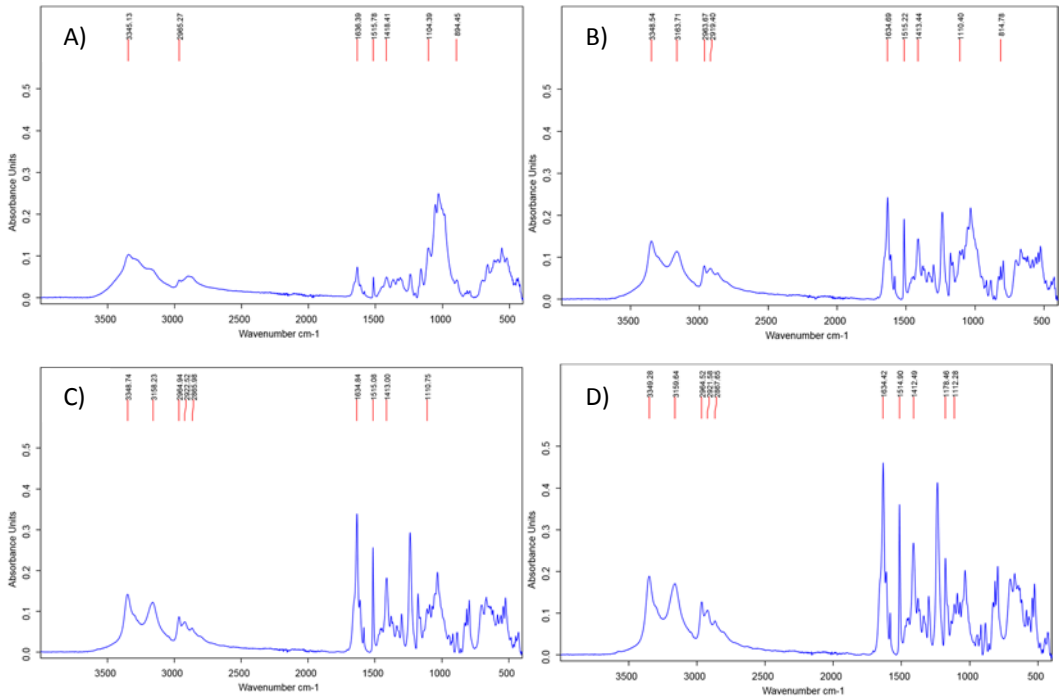

Figure 6.7: ATR FTIR Spectra of standard calibration mixtures, A) 5% atenolol, B) 25% atenolol, C) 50% atenolol and D) 75% atenolol

Figure 6.7 shows selected spectra for the different calibration mixes and the change in key peaks for atenolol and MCC, as the concentration varied.

Table 6.1 summarises the characteristic peaks for atenolol and MCC and the presence of them in the calibration mixtures.

Table 6.1: Summary of characteristic atenolol and MCC peaks in calibration mixtures

Calibration Mixture	Characteristic Peaks (cm-1)									
	Atenolol							MCC		
	3347	**3159**	**2966**	**2866**	**1633**	**1515**	**1412**	**3328**	**1104**	**897**
100% Atenolol: 0% MCC	✓	✓	✓	✓	✓	✓	✓	×	×	×
75% Atenolol: 25% MCC	✓	✓	✓	✓	✓	✓	✓	~	~	×
60% Atenolol: 40% MCC	✓	✓	✓	✓	✓	✓	✓	~	~	×
50% Atenolol: 50% MCC	✓	✓	✓	✓	✓	✓	✓	~	~	×
40% Atenolol: 60% MCC	✓	✓	✓	✓	✓	✓	✓	~	~	×
30% Atenolol: 70% MCC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
25% Atenolol: 75% MCC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
20% Atenolol: 80% MCC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
10% Atenolol: 90% MCC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5% Atenolol: 95% MCC	~	×	×	×	✓	✓	✓	✓	✓	✓
0% Atenolol: 100% MCC	×	×	×	×	×	×	×	✓	✓	✓

From the table, it can be shown that it is possible to detect atenolol at 10% within an excipient mix. In addition, atenolol can be potentially detected at 5%, as some characteristic peaks are present.

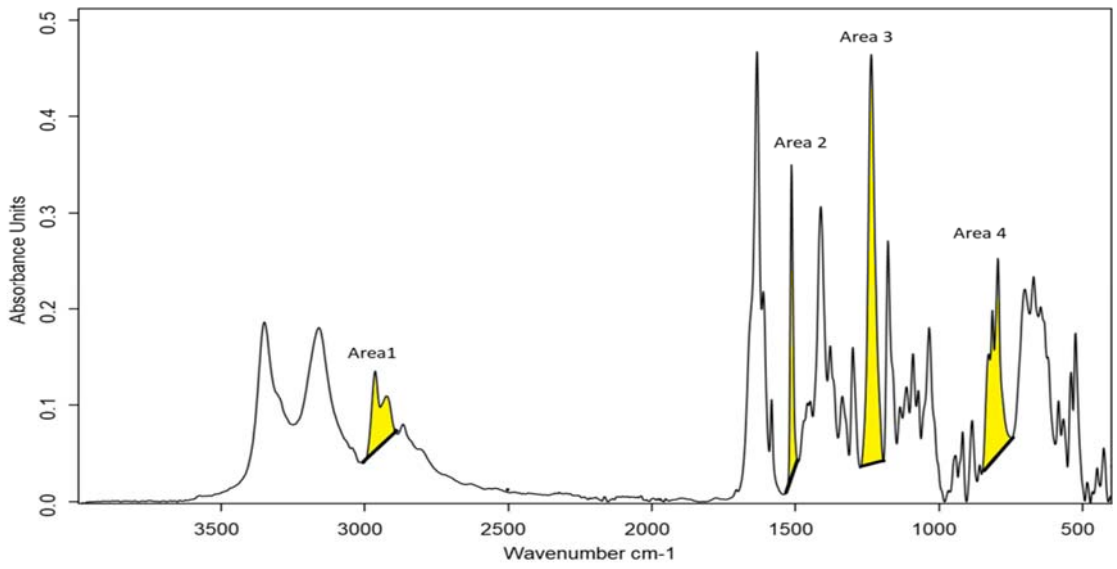

Figure 6.8: ATR FTIR spectrum of atenolol with integration areas highlighted

A total of 12 different calibration plots were calculated from the mean integrated peak areas (Figure 6.8) over the ranges of 1) 3009 – 2893cm^{-1}, 2) 1530 – 1490cm^{-1}, 3) 1272 – 1193cm^{-1} and 4) 858 – 758cm^{-1}, using the different integration methods.

The calibration plots for the various methods and peak areas gave the following equations of a line and R^2 values.

- Method A:
  - Area 1) $y = 0.0715x + 4.5616$ $R^2 = 0.8359$
  - Area 2) $y = 0.0432x + 0.5586$ $R^2 = 0.9242$
  - Area 3) $y = 13.59x + 1.9101$ $R^2 = 0.9479$
  - Area 4) $y = 0.1195x + 0.2017$ $R^2 = 0.9709$

- Method B:
  - Area 1) $y = 0.0414x + 0.2586$ $R^2 = 0.9026$
  - Area 2) $y = 0.0336x + 0.4782$ $R^2 = 0.9080$
  - Area 3) $y = 11.075x + 1.3348$ $R^2 = 0.9261$
  - Area 4) $y = 0.0775x + 0.6067$ $R^2 = 0.9327$

- Method K:
  - Area 1) $y = 0.0008x + 0.0270$ $R^2 = 0.9283$
  - Area 2) $y = 0.0031x + 0.0432$ $R^2 = 0.9102$
  - Area 3) $y = 0.4629x + 0.056$ $R^2 = 0.9256$
  - Area 4) $y = 0.0020x - 0.0191$ $R^2 = 0.9323$

Table 6.2 ranks the equations based on the R^2 values for the different integration methods.

Table 6.2: Integration methods ranked according to R^2 values for atenolol

Integration Method and Peak Area	**R^2 Value**	**Rank**
A1	0.8359	12
A2	0.9242	8
A3	0.9479	2
A4	0.9709	1
B1	0.9026	11
B2	0.9080	10
B3	0.9261	6
B4	0.9327	3
K1	0.9283	5
K2	0.9102	9
K3	0.9258	7
K4	0.9323	4

Analysing the R^2 values in Table 6.2, integration method A and peak area 4 (Figure 6.9) yielded the best results, the R^2 value is the closest to one.

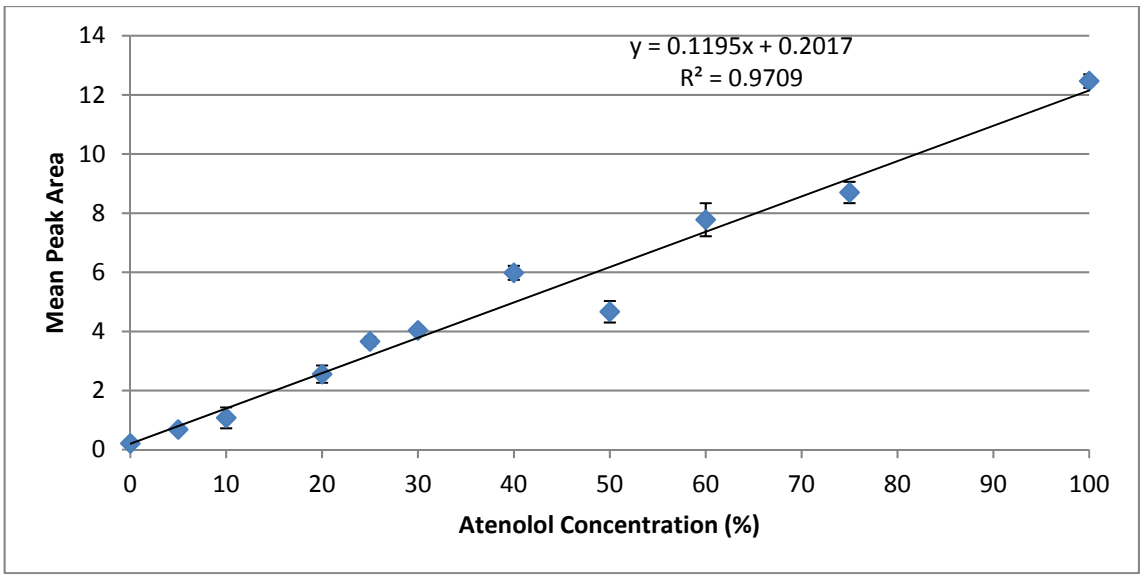

Figure 6.9: Calibration plot using integration method A and peak area 4 for atenolol with error bars

Rearranging the equation of the line (Figure 6.9) for integration method A, peak area 4 it is possible to calculate the concentration of atenolol in a tablet.

The equation of the line is: y= 0.1194803x + 0.2017352

Rearrange to give x, x = (y - 0.2017352) / 0.1194803

Where x is the concentration of atenolol and y is the mean integral.

6.2.1.2 *Quantification of API within a tablet*

Table 6.3 summarises the quantification results for the UK atenolol tablets using ATR FTIR and are shown graphically in Figure 6.10. Overall most tablets are in the range as outlined by the EMA (90 – 110%). The tablets At/UK/1, At/UK/2 and At/UK/3 generally all fall below this threshold, suggesting that the methodology does not work for this formulation. Further work is needed to determine if formulation of tablets affects the quantification. At/UK/7 also is outside the EMA range, but within the FDA range (80 – 125%) for a higher calculated amount of API.

Table 6.3: Quantification results for UK atenolol tablets calculated by ATR FTIR

Tablet	Country	Theoretical Conc	Mean Integral	Weight of Tablet (g)	Calculated Amount (%)	Calculated Amount (mg)
At/UK/1	UK	25mg	3.4674	0.1057	16.37	17.30
At/UK/2	UK	50mg	3.676	0.2167	17.42	37.74
At/UK/3	UK	100mg	3.6548	0.4231	17.31	73.24
At/UK/4	UK	25mg	4.491	0.1097	21.50	23.59
At/UK/5	UK	50mg	4.4708	0.2189	21.40	46.85
At/UK/6	UK	100mg	4.6472	0.4286	22.29	95.51
At/UK/7	UK	50mg	5.6488	0.2066	27.31	56.41

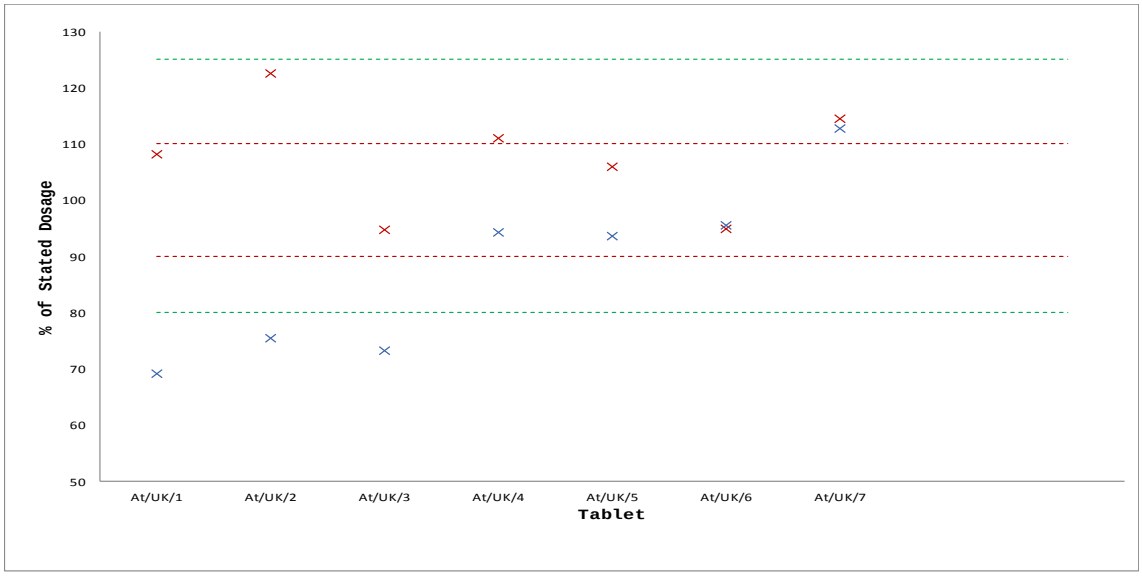

Figure 6.10: Percentage of stated dosage of UK atenolol Tablets as calculated by UV (X) and ATR FTIR (X) with EMA (90-110%) (--) and FDA (80-125%) (--) limits

The quantification results for the Indian, Pakistani, Saudi Arabian and Nepalese atenolol tablets using ATR FTIR are summarised in Table 6.4 and are shown graphically in Figure 6.11 for the Indian tablets and Figure 6.12 for the Pakistani, Saudi Arabian and Nepalese tablets. Overall most tablets are outside range as outlined by the EMA (90 – 110%), this is quite concerning as it may mean most of these tablets are substandard. Further work is needed to determine if these tablets are substandard. The At/NEP/1 sample is in the acceptable range for the EMA.

Table 6.4: Quantification Results for Indian Atenolol Tablets as calculated by ATR FTIR

Tablet	Country	Theoretical Conc	Mean Integral	Weight of Tablet (g)	Calculated Amount (%)	Calculated Amount (mg)
At/IND/1	India	25mg	3.0774	0.1883	14.42	27.14
At/IND/2	India	25mg	2.4758	0.1856	11.40	21.16
At/IND/5	India	50mg	2.7508	0.1827	12.78	23.35
At/IND/6	India	50mg	3.4662	0.1839	16.36	30.09
At/IND/7	India	50mg	3.2022	0.1837	15.04	27.63
At/IND/8	India	50mg	3.5206	0.1878	16.64	31.25
At/IND/9	India	50mg	3.5492	0.1877	16.78	31.50
At/IND/10	India	50mg	3.5966	0.1828	17.02	31.11
At/IND/11	India	25mg	2.605	0.1865	12.05	22.47
At/IND/12	India	50mg	3.2008	0.1843	15.03	27.71
At/IND/13	India	50mg	3.3318	0.1878	15.69	29.47
At/IND/14	India	50mg	2.1786	0.1943	9.91	19.26
At/IND/15	India	50mg	3.9304	0.1903	18.69	35.57
At/IND/16	India	50mg	3.7428	0.1899	17.75	33.71
At/IND/17	India	25mg	4.5005	0.1097	21.55	23.64
At/IND/18	India	50mg	3.7692	0.1936	17.88	34.62
At/PAK/1	Pakistan	50mg	4.3556	0.1524	20.82	31.73
AT/PAK/2	Pakistan	100mg	3.4882	0.2925	16.48	48.19
At/SA/1	Saudi Arabia	50mg	5.888	0.2041	28.51	58.18
At/SA/2	Saudi Arabia	50mg	5.4638	0.2114	26.38	55.76
At/SA/3	Saudi Arabia	100mg	1.7088	0.4141	7.55	31.29
At/SA/4	Saudi Arabia	100mg	1.5238	0.3994	6.63	26.47
At/SA/5	Saudi Arabia	100mg	1.8138	0.4111	8.08	33.22
At/NEP/1	Nepal	50mg	4.7278	0.2017	22.69	45.76
At/NEP/2	Nepal	50mg	2.4236	0.1884	11.14	20.98
At/NEP/3	Nepal	50mg	3.0868	0.1837	14.46	26.57

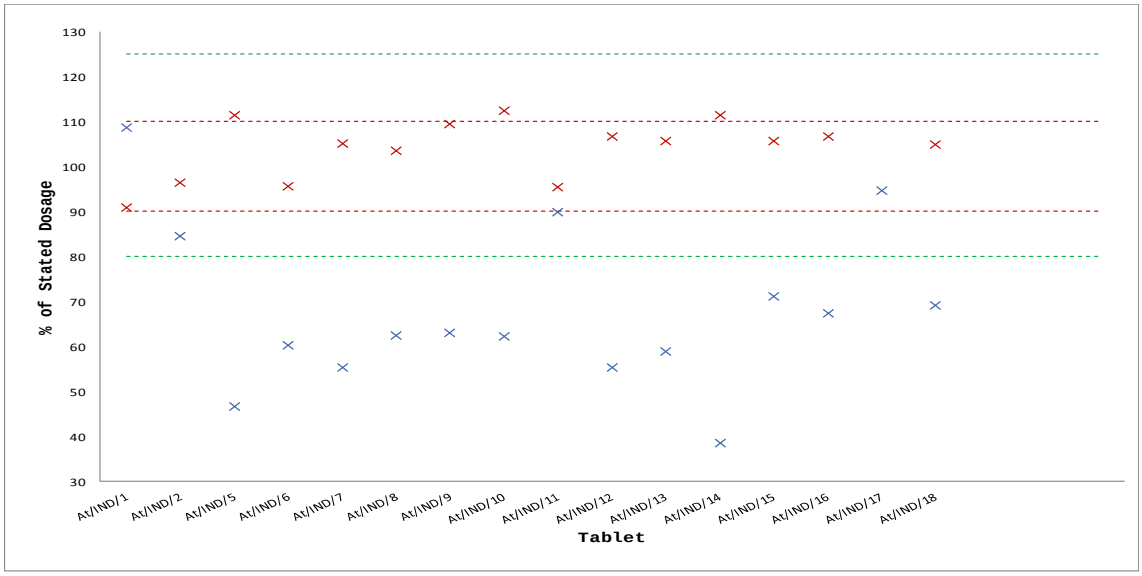

Figure 6.11: Percentage of stated dosage of Indian atenolol tablets as calculated by UV (X) and ATR FTIR (X) with EMA (90-110%) (--) and FDA (80-125%) (--) limits

In general, the trend is to underestimate the level of atenolol within the tablet, it is interesting to note that the ATR FTIR results for the four Indian samples (At/IND/1, AT/IND/2, At/IND/11 and At/IND/17) do not fit this trend. On closer inspection of the tablet weights and dosage level of the API, these four tablets are stated to contain 25mg of the API, but have a similar tablet weight to the 50mg tablets. This suggests that the API is present in a lower ratio to excipients for 25mg than the 50mg tablets. This 'dilution' of the API within the tablet may prevent agglomeration of the API; this will be discussed further in this chapter. The results for the Indian 25mg are not replicated for the UK tablets, as the tablet weight varies according to dose.

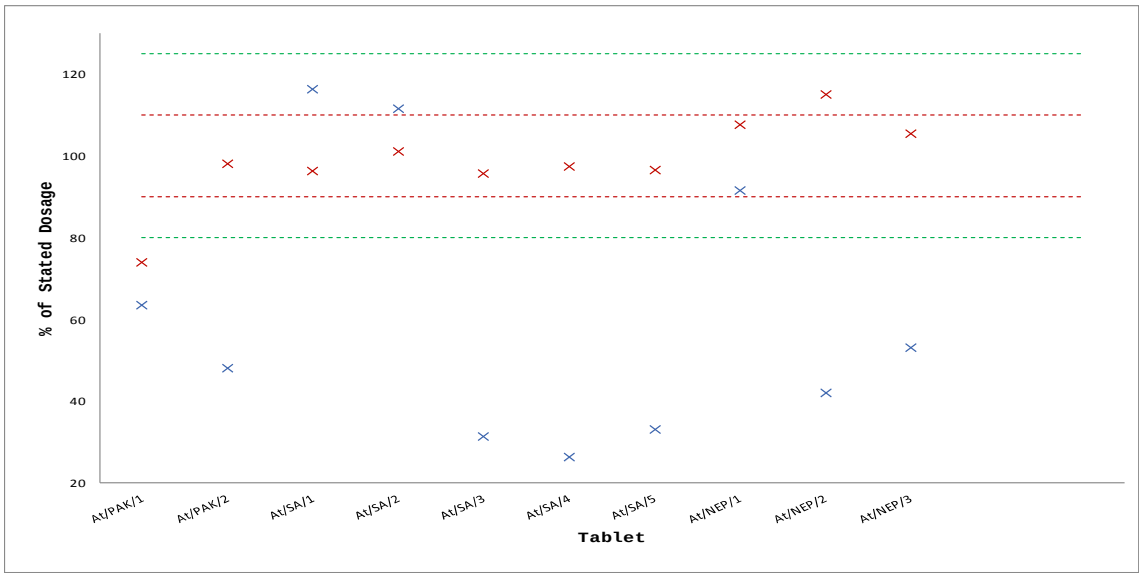

Figure 6.12: Percentage of stated dosage of Pakistani, Saudi Arabian and Nepalese atenolol tablets as calculated by UV (X) and ATR FTIR (X) EMA (90-110%) (--) and FDA (80-125%) (--) limits

Overall, the results for quantification are low for the majority of tablets from the different countries; this may be due to excipients interacting with the chosen peak range to quantify from or it may stem from poor mixing (not quite achieving a maximum degree of randomness) and potential issues with agglomeration of particles. Salari and Young (1998) discussed that not attaining a 'perfect mixture' or as close to would affect quantification of powders. It is possible that atenolol agglomerates and therefore did not achieve a 'perfect mixture' or one, which had a high degree of randomness.

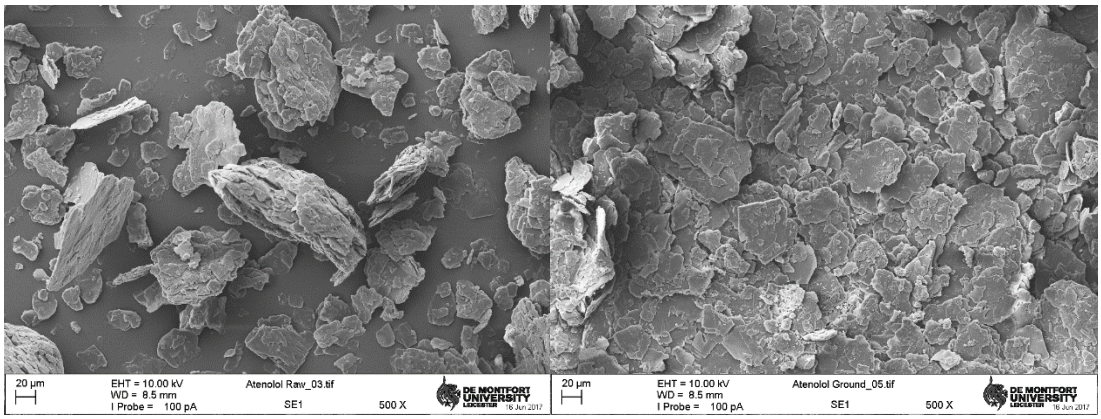

Figure 6.13: SEM Images for atenolol A) raw and B) ground.

The analytical reference standard for atenolol was analysed by SEM (Figure 6.13) in both its raw and ground format. From the images, it is clear that once the atenolol is ground using a pestle and mortar it sticks together and agglomerates. This physical attribute may have influenced the calibration plot and may/ may not affect the quantification of the API in a tablet form. Quantification data was calculated using the equation of the line for other peak areas and the results still gave a lower amount of API than expected.

6.2.2 Metformin Hydrochloride

6.2.2.1 *Calibration mixtures*

Calibration mixtures for the quantification of metformin hydrochloride in a single tablet were prepared as detailed in Chapter 4.3.1. Calibration mixtures of metformin hydrochloride and maize starch were analysed. Characteristic peaks for metformin hydrochloride were observed for the calibration mixtures (Table 6.5).

Table 6.5: Summary of characteristic metformin and maize starch peaks in calibration mixtures

Calibration Mixture	Characteristic Peaks (cm-1)									
	Metformin hydrochloride								Maize starch (MS)	
	3367	3150	1623	1417	1165	1061	581	420	3288	996
100% Metformin: 0% MS	✓	✓	✓	✓	✓	✓	✓	✓	×	×
90% Metformin: 10% MS	✓	✓	✓	✓	✓	✓	✓	✓	×	×
80% Metformin: 20% MS	✓	✓	✓	✓	✓	✓	✓	✓	×	×
70% Metformin: 30% MS	✓	✓	✓	✓	✓	✓	✓	✓	×	×
60% Metformin: 40% MS	✓	✓	✓	✓	✓	✓	✓	✓	×	×
50% Metformin: 50% MS	✓	✓	✓	✓	✓	~	✓	✓	×	~
40% Metformin: 60% MS	✓	✓	✓	✓	~	~	✓	✓	×	✓
30% Metformin: 70% MS	✓	✓	✓	✓	✓	~	✓	✓	×	✓
20% Metformin: 80% MS	✓	✓	✓	~	~	~	✓	✓	~	✓
10% Metformin: 90% MS	×	×	×	×	×	~	~	×	✓	✓
0% Metformin: 100% MS	×	×	×	×	×	×	×	×	✓	✓

From the table, it can be shown that it is possible to detect metformin hydrochloride at concentrations >10% within an excipient mix.

Four peak areas characteristic to metformin hydrochloride were integrated using the Opus software. These were Area 1) 1648 –1605cm^{-1}, Area 2) 1605 – 1501cm^{-1}, Area 3) 1501 – 1432cm^{-1} and Area 4) 1432 – 1341cm^{-1} (Figure 6.14). The peak areas were integrated using the different integration methods.

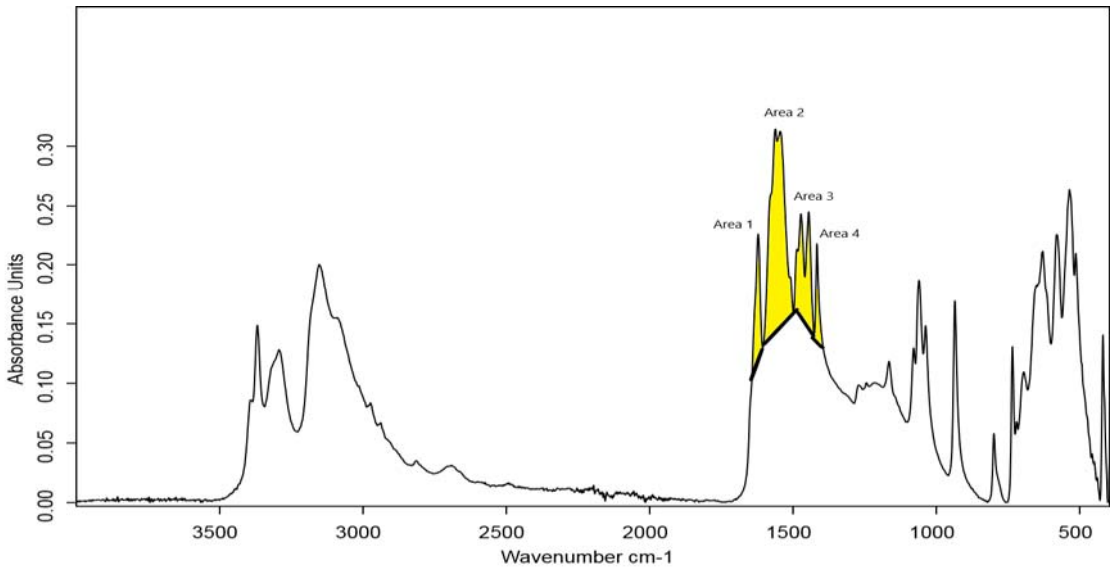

Figure 6.14: ATR FTIR spectrum of metformin hydrochloride with integration areas highlighted

The 12 calibration plots for the various integration methods and peak ranges gave the following equations of a line and R^2 values.

- Method A:
  - Area 1) $y = 0.0489x + 0.2156$ $R^2 = 0.8610$
  - Area 2) $y = 0.1995x - 1.691$ $R^2 = 0.8682$
  - Area 3) $y = 0.1034x - 0.6414$ $R^2 = 0.8050$
  - Area 4) $y = 0.0427x + 0.2455$ $R^2 = 0.6791$

- Method B:
  - Area 1) $y = 0.0147x - 0.0744$ $R^2 = 0.8740$
  - Area 2) $y = 0.0823x - 0.9235$ $R^2 = 0.8785$
  - Area 3) $y = 0.0298x - 0.2660$ $R^2 = 0.8529$
  - Area 4) $y = 0.0076x - 0.1088$ $R^2 = 0.8587$

- Method K:
  - Area 1) $y = 0.0009x - 0.0066$ $R^2 = 0.8805$
  - Area 2) $y = 0.0015x - 0.0115$ $R^2 = 0.8915$
  - Area 3) $y = 0.0007x - 0.0060$ $R^2 = 0.8452$
  - Area 4) $y = 0.0005x - 0.0050$ $R^2 = 0.8762$

Table 6.6: Integration methods ranked accorded to R^2 values for metformin hydrochloride

Integration Method and Peak Area	R^2 Value	Rank
A1	0.8610	7
A2	0.8682	6
A3	0.8050	11
A4	0.6791	12
B1	0.8740	5
B2	0.8785	3
B3	0.8529	9
B4	0.8587	8
K1	0.8805	2
K2	0.8915	1
K3	0.8452	10
K4	0.8762	4

Table 6.6 ranks the R^2 values according to their value, those that are closest to one are ranked highest. Analysing the R^2 values integration method K and peak area 2 yielded the best results, with the R^2 value is the closest to one.

Rearranging the equation of the line for integration method K, peak area 2 it is possible to calculate the concentration of metformin hydrochloride in a tablet.

The equation of the line is: $y = 0.0015x - 0.0115$

Rearrange to give x, x = (y + 0.0115) / 0.0015)

Where x is the concentration of metformin hydrochloride and y is the mean integral.

6.2.2.2 *Quantification of API within a tablet*

Table 6.7 summarises the quantification results for the UK metformin hydrochloride tablets using ATR FTIR and are shown graphically in Figure 6.15. Overall, the tablets are outside range as

outlined by the FDA (80 – 125%), this could indicate substandard medicines, or there may be an issue with the calibration method, as the R^2 value for the calibration plot showed poor linearity.

Table 6.7: Quantification results for UK metformin hydrochloride tablets as calculated by ATR FTIR

Tablet	Country	Theoretical Conc (mg)	Mean Integral	Weight of Tablet (g)	Calculated Amount (%)	Calculated Amount (mg)
Met/UK/1	UK	500	0.1574	0.5899	112.60	664.23
Met/UK/2	UK	850	0.149	0.9928	107.00	1062.30

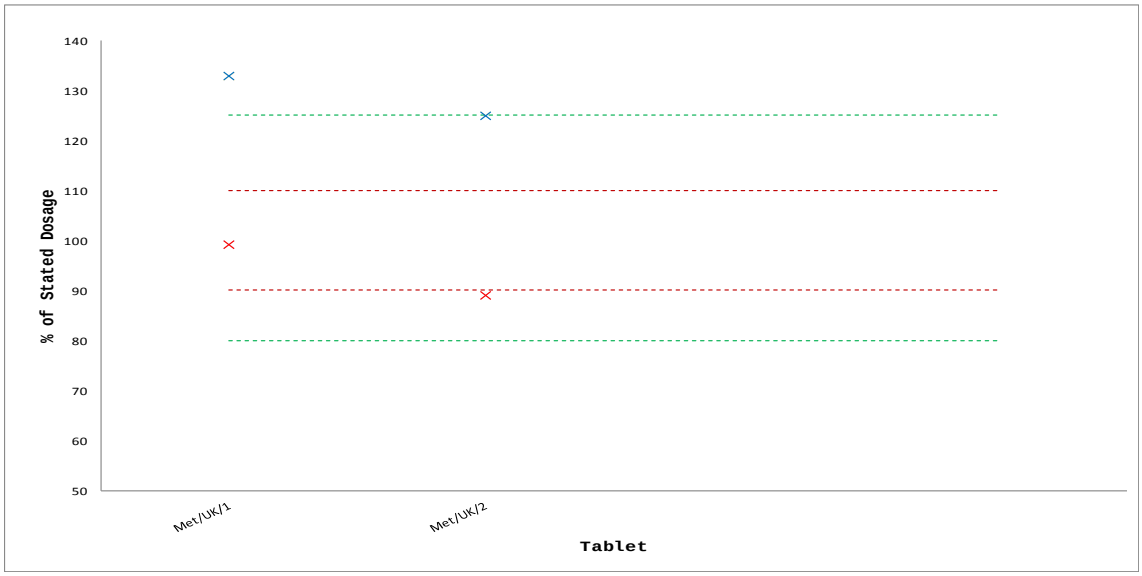

Figure 6.15: Percentage of stated dosage of UK metformin hydrochloride tablets as calculated by UV (X) and ATR FTIR (X) with EMA (90-110%) (--) and FDA (80-125%) (--) limits

Table 6.8 summarises the quantification results for the Indian and Saudi Arabian metformin hydrochloride tablets using ATR FTIR and are shown graphically in Figure 6.16. Overall, the tablets are outside range as outlined by the EMA (90 – 110 %), this could indicate substandard medicines. There may be an issue with the calibration method, as the R^2 value for the calibration plot showed poor linearity, or the equation chosen, but the results for a range of UV analyses were inside the acceptable range.

Table 6.8: Quantification results for Indian and Saudi Arabian metformin hydrochloride tablets as calculated by ATR FTIR

Tablet	Country	Theoretical Conc (mg)	Mean Integral	Weight of Tablet (g)	Calculated Amount (%)	Calculated Amount (mg)
Met/IND/1	India	250	0.0846	0.2946	64.07	188.74
Met/IND/2	India	500	0.1154	0.5984	84.60	506.25
Met/IND/3	India	500	0.1654	0.7085	117.93	835.56
Met/IND/4	India	850	0.1356	1.0161	98.07	996.46
Met/IND/5	India	850	0.1674	1.1285	119.27	1345.92
Met/IND/6	India	500	0.1104	1.0132	81.27	823.39
Met/IND/8	India	500	0.1174	0.6765	85.93	581.34
Met/IND/9	India	500	0.1352	0.6731	97.80	658.29
Met/IND/10	India	500	0.1434	0.5842	103.27	603.28
Met/IND/11	India	500	0.1464	0.7417	105.27	780.76
Met/IND/12	India	400	0.082	0.5883	62.33	366.71
Met/IND/13	India	400	0.1526	0.5396	109.40	590.32
Met/IND/14	India	500	0.1284	0.6389	93.27	595.88
Met/IND/15	India	500	0.1524	0.6448	109.27	704.55
Met/IND/16	India	1000	0.139	1.3401	100.33	1344.57
Met/IND/17	India	500	0.1584	0.5233	113.27	592.72
Met/SA/1	Saudi Arabia	500	0.1366	0.5396	98.73	532.77

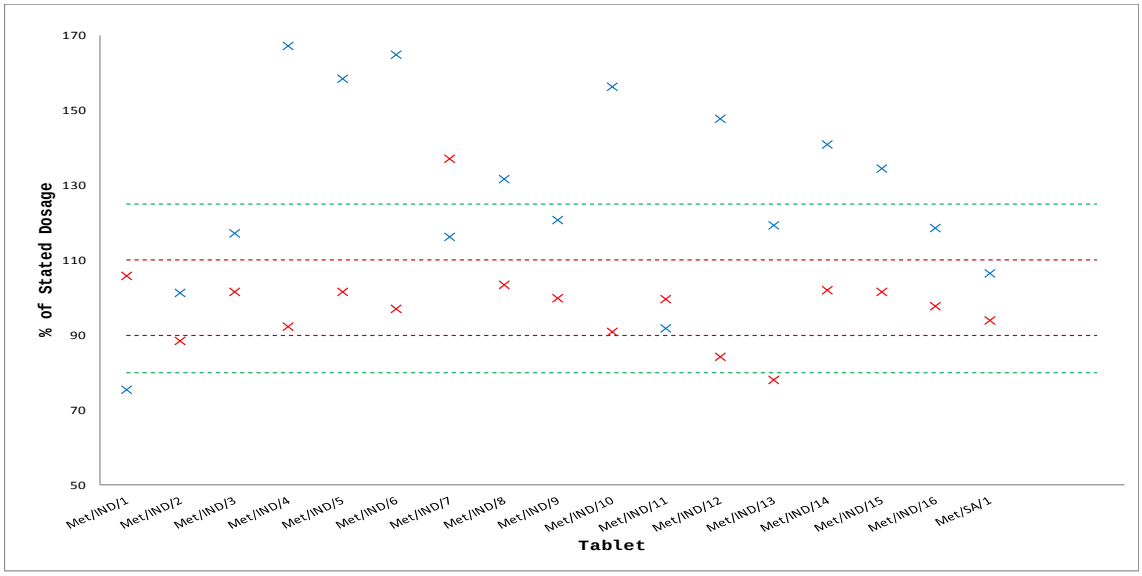

Figure 6.16: Percentage of stated dosage of Indian and Saudi Arabian metformin hydrochloride tablets as calculated by UV (X) and ATR FTIR (X) EMA (90-110%) (--) and FDA (80-125%) (- -) limits

Overall, the results for quantification are high for the majority of tablets from the different countries; this may be due to excipients interacting with the chosen peak to quantify from or it may stem from poor mixing and/or issues with agglomeration of particles. Salari and Young (1998) discussed that not attaining a 'perfect mixture' or as close to would affect quantification of powders. It is possible that either the API or excipient agglomerates and therefore did not achieve a 'perfect mixture'. Using the equation of a line for other integration methods and peak areas, the quantification results for metformin hydrochloride still tended to be on the high side of the acceptable limits. Further work is required to investigate the choice of peak area for quantification.

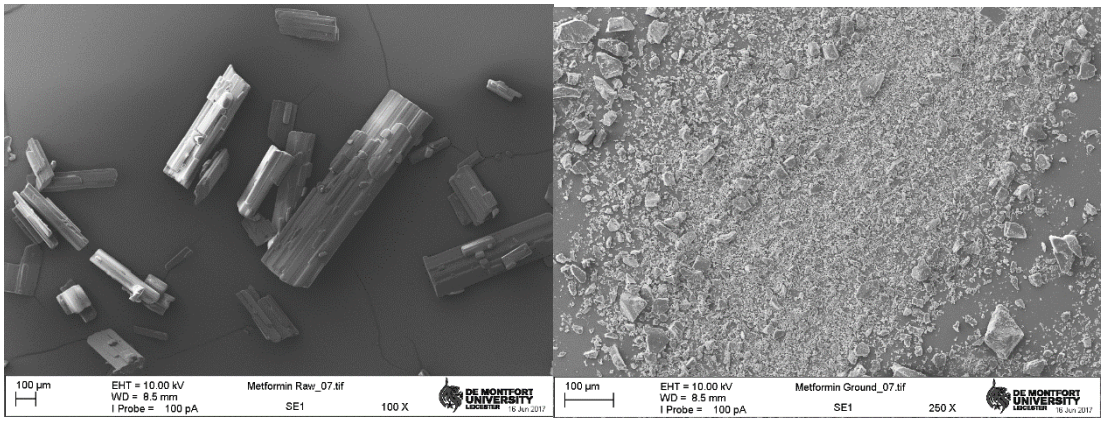

Figure 6.17: SEM images for metformin hydrochloride A) raw and B) ground

The reference standard for metformin hydrochloride was analysed by SEM (Figure 6.17) in both its raw and ground format. From the images, it can be inferred that metformin hydrochloride is a crystalline material and once ground using a pestle and mortar it is a free-flowing powder. It is therefore important to analyse the excipient (maize starch) (Figure 6.18) and a mixture of API and excipient (Figure 6.19).

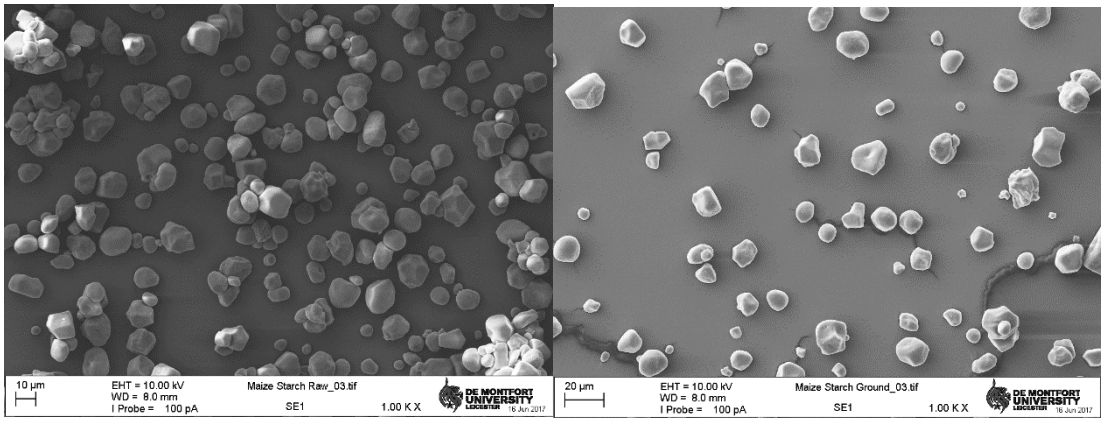

Figure 6.18: SEM images for maize starch A) raw and B) ground

The images in Figure 6.18 show that maize starch is a free flowing powder both when it is raw and ground format. The particle size is not uniform, even after grinding which could indicate potential reasons for the poor quantification ATR FTIR results.

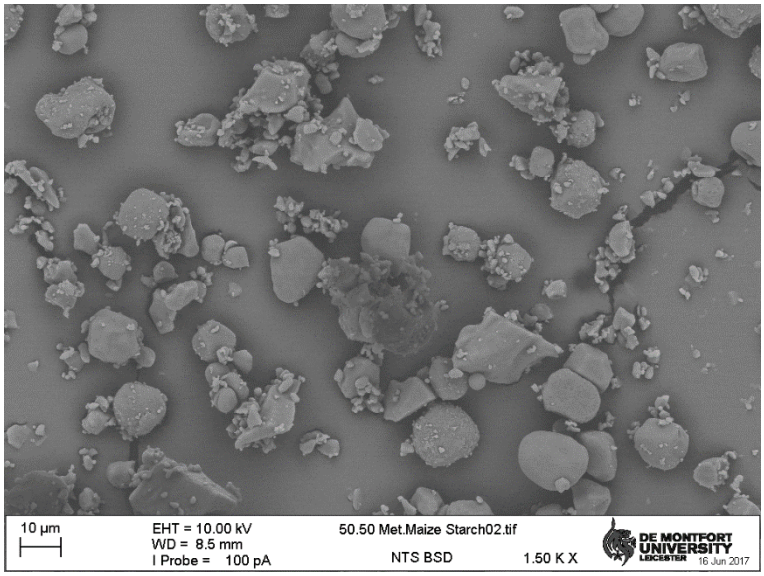

Figure 6.19: SEM images for 50:50 metformin hydrochloride: maize starch.

The image for the 50:50 calibration mix shows a good distribution of maize starch (the large particles); however, the distribution of metformin hydrochloride does not appear to be uniform,

as it is not distributed evenly with the excipient. This may account for the poor linearity of the calibration plots and the apparent variation in concentration of metformin hydrochloride.

6.3 Raman spectroscopy

6.3.1 Atenolol

Calibration mixtures of atenolol and lactose were prepared over the range of 0 – 100% at 10% intervals. The calibration mixes were different to those used for ATR FTIR, as MCC is known to fluoresce and for this reason, lactose was selected. A calibration plot was calculated from the peak heights of 366cm^{-1}. The peaks that were not masked by the excipient were severely limited and resulting in just one peak being selected for quantification.

The calibration plot gave a significantly low R^2 value (0.0203), suggesting that the data for atenolol and lactose was poor. Further work is required to investigate other excipients with the API, in order to determine if quantification of an API using Raman is viable.

6.3.2 Paracetamol

Calibration mixtures of paracetamol and maize starch were prepared over the range of 0 – 100% at 10% intervals to see if the poor results for atenolol were API or technique related. Calibration plots were calculated from the peak heights of (1) 212cm^{-1}, 2) 650cm^{-1}, 3) 1612cm^{-1} and 4) 1650cm^{-1}).

The calibration plots for the various peaks gave the following equations of a line and R^2 values.

- Peak 1) $y = 0.0190x + 0.0767$ $R^2 = 0.8620$
- Peak 2) $y = 0.0422x - 0.1242$ $R^2 = 0.8919$
- Peak 3) $y = 0.0952x - 0.4739$ $R^2 = 0.8975$
- Peak 4) $y = 0.0759x - 0.3498$ $R^2 = 0.8937$

The R2 values for the paracetamol calibration are low and suggest poor linearity of the data. Further work is needed to investigate this.

6.4 EDX spectroscopy

As most of the tablets showed similar morphology to one another using SEM, the cross sections of the tablets were mapped and quantified using EDX. Many APIs and excipients contain marker elements, for example phosphorous, chlorine, titanium and magnesium, which can be detected by EDX spectroscopy and used to provide an elemental fingerprint of a particular tablet formulation.

From this fingerprint, the nature of some excipients and possibly the API/s can be inferred. Figure 6.20 shows an overlaid elemental map for a cross section of an ibuprofen tablet, mapping data can be overlaid and false coloured to give the spatial distribution of elements. From the composite image, several observations can be made; the tablet has a clear distinct coating (blue), suggesting the use of titanium dioxide in the formulation, the disintegrant croscarmellose sodium is present as indicated by the red areas and the manufacturer uses an iron oxide to print the brand name on the tablet (pink).

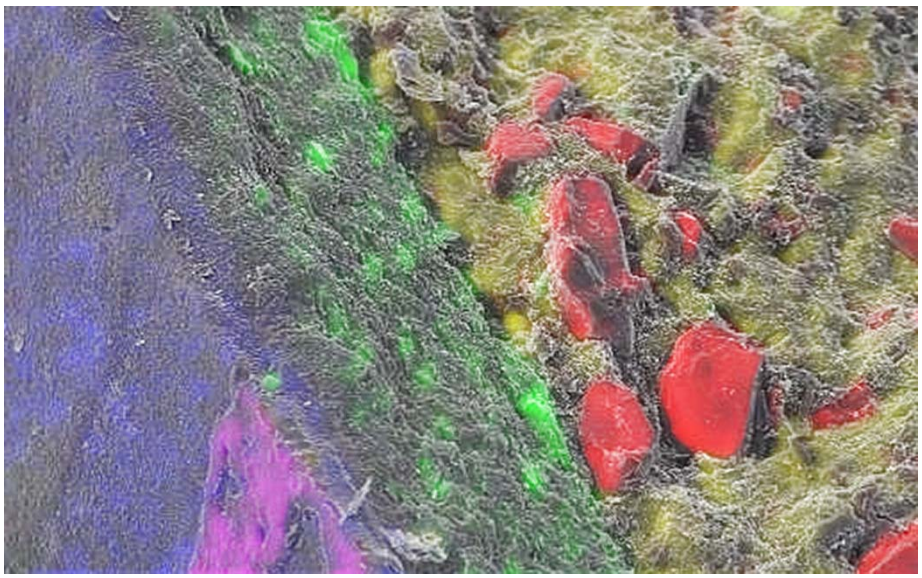

Figure 6.20: Composite image of elemental maps for a cross section of an ibuprofen tablet (titanium (blue), magnesium/ silicon (green), carbon (yellow), sodium (red) and iron (pink))

For the purpose of this study, the quantification data for carbon and oxygen is reported, but is not interpreted as these elements are present in all organic molecules and we are interested in marker elements.

6.4.1 Atenolol

6.4.1.1 *UK Tablets*

The EDX data for the samples At/UK/1, At/UK/2 and At/UK/3 can be summarised in Table 6.9. From the quantification data, the different dosages of the At/UK/1 -3 tablets are of an identical elemental composition, with similarities in the quantification data. The AT/UK/3 tablet compromised of a higher nitrogen concentration and lower magnesium and aluminium concentration. The formulation for At/UK/4 - 6 samples is similar to that of the At/UK/1 -3 samples with the exception of the absence of nitrogen and the presence of titanium. One of the reasons for the variation in nitrogen concentration maybe due to nitrogen being a hard element to

quantify especially in the presence of titanium, this is because the Kα peaks for nitrogen and titanium overlap.

Table 6.9: Comparison of EDX quantification data for At/UK/1 -3 tablets

Element	**Concentration (%)**		
	At/UK/1	**At/UK/2**	**At/UK/3**
Carbon	49.54	47.79	50.10
Nitrogen	7.71	7.43	8.20
Oxygen	39.34	40.98	38.88
Sodium	0.17	0.19	0.14
Magnesium	2.62	2.98	2.21
Aluminium	0.27	0.25	0.11
Silicon	0.26	0.29	0.26
Chlorine	0.05	0.06	0.05
Calcium	0.02	0.03	0.03

From the mapping data, the possibility of carbon, oxygen and magnesium being present in the outer layer of the coating, suggests that opacifiers were not used in the formulation of this brand of tablet. Distinct regions of magnesium particles in the tablet core can be observed (Figure 6.21).

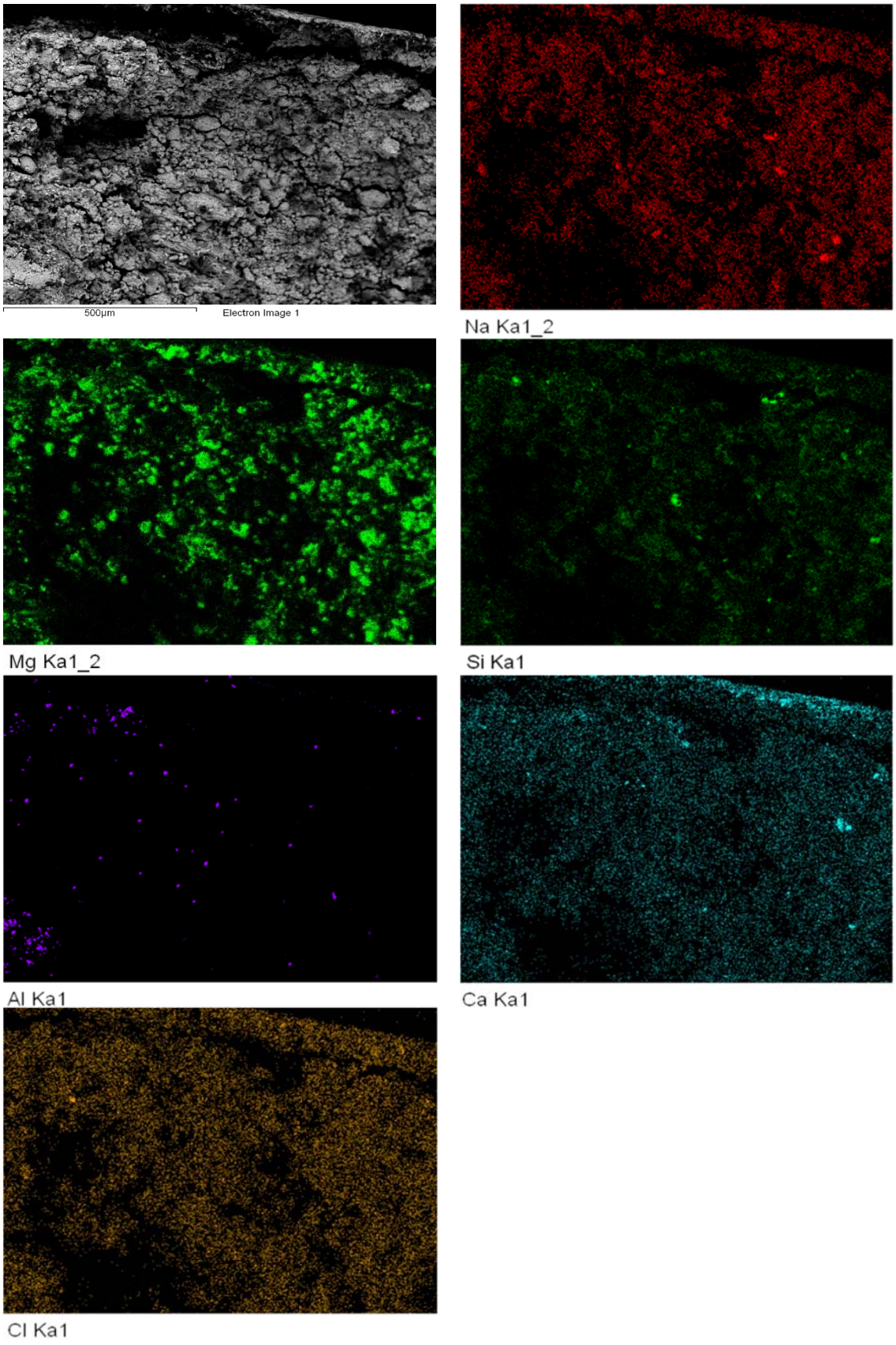

Figure 6.21: EDX Map for At/UK/3

The EDX data for the samples At/UK/4, At/UK/5 and At/UK/6 is summarised in Table 6.10. From the quantification data, the different dosages of tablets from the same manufacturer are a similar elemental composition.

Table 6.10: Comparison of EDX quantification data for At/UK/4 -6 tablets

Element	**Concentration (%)**		
	At/UK/4	**At/UK/5**	**At/UK/6**
Carbon	58.26	57.95	56.98
Oxygen	37.88	39.23	39.26
Sodium	0.17	0.14	0.19
Magnesium	2.46	2.36	2.51
Aluminium	0.82	0.04	0.52
Silicon	0.27	0.21	0.41
Chlorine	0.07	0.05	0.07
Calcium	0.03	0.02	0.03
Titanium	0.04		0.03

From the mapping data, there is an absence of a clear element present in the coating; the At/UK/6 map identifies the possibility of carbon and magnesium present in the outer layer of the tablet (Figure 6.22). The presence of titanium in the tablet core is interesting, as titanium (titanium dioxide) is normally found on the coating of tablets. The excipient titanium dioxide is more commonly used as a whitening agent. The At/UK/4 and At/UK/6 tablets contained a higher level of aluminium, which was distributed as distinct regions in the tablet core; the At/UK/6 tablet also had a distinct region of silicon.

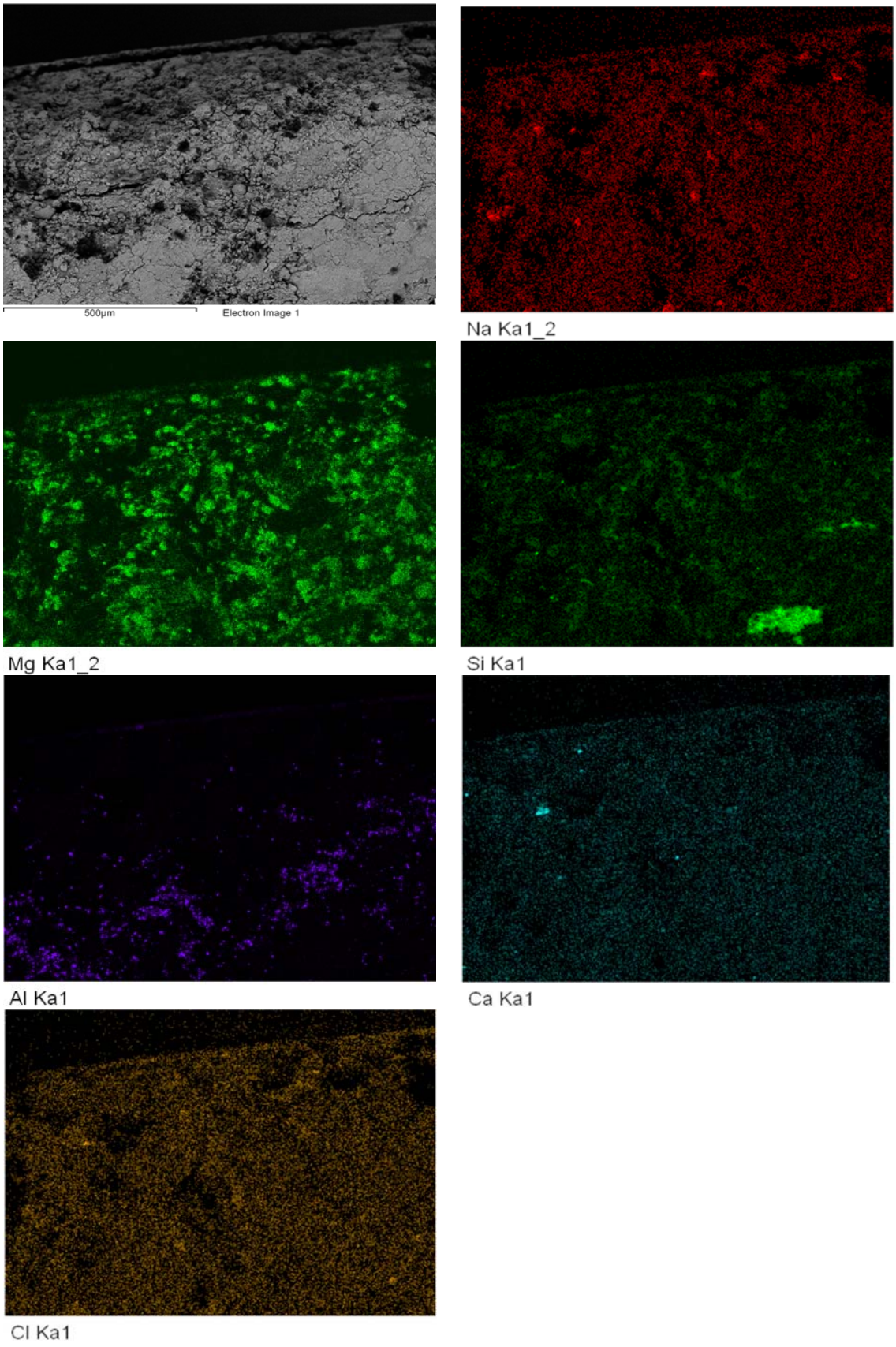

Figure 6.22: EDX Map of At/UK/6

The EDX data for the At/UK/7 tablet is summarised in Table 6.11. From the quantification data, the At/UK/7 tablet consists of fewer elements, suggesting that less excipients are used in the formulation. There is a higher concentration of carbon present in the sample, though this may be relative as there is an absence of nitrogen in the tablet cross section. The magnesium content indicates the presence of magnesium carbonate and magnesium stearate, as indicated by the patient information leaflet.

Table 6.11: EDX quantification data for At/UK/7 tablet

Element	**Concentration (%)**
Carbon	58.53
Oxygen	38.22
Magnesium	3.20
Titanium	0.05

The mapping data shows a clear layer of titanium in the coating, suggesting that titanium dioxide was used as an excipient to 'whiten' the coating (Figure 6.23).

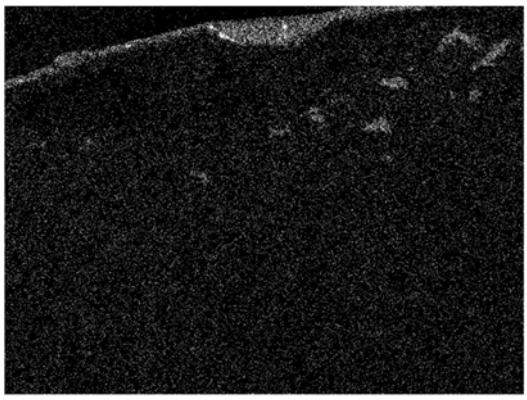

Figure 6.23: EDX map showing distribution of titanium for At/UK/7

6.4.1.2 *Indian Tablets*

The EDX data for At/IND/1 – 2 and At/IND/4 – 10 is summarised in Table 6.12. Elemental composition and quantification data can be inferred from the data. Elemental analysis identified the presence of aluminium in only two tablets (At/IND/1 and At/IND/8) and phosphorous in three tablets (At/IND/1, At/IND/2 and At/IND/5).

Table 6.12: Comparison of EDX quantification data for At/IND/1-2 and At/IND/4-10

Element	**Concentration (%)**							
	At/IND/1	**At/IND/2**	**At/IND/4**	**At/IND/5**	**At/IND/7**	**At/IND/8**	**At/IND/9**	**At/IND/10**
Carbon	52.30	52.26	53.33	54.21	54.64	54.30	54.39	55.15
Nitrogen	8.39	8.76	11.93	12.37	11.46	11.5	13.69	11.99
Oxygen	36.33	36.24	33.30	32.09	32.59	32.53	30.42	31.58
Sodium	0.23	0.18	0.10	0.10	0.17	0.18	0.22	0.17
Magnesium	0.33	0.28	0.33	0.22	0.26	0.34	0.285	0.22
Aluminium	0.03					0.05		
Silicon	0.61	0.58	0.73	0.55	0.57	0.68	0.61	0.52
Phosphorous	0.83	0.79		0.20				
Chlorine	0.04	0.04	0.04	0.03	0.03	0.03	0.07	0.06
Calcium	0.91	0.89	0.24	0.22	0.29	0.23	0.32	0.31

At/IND/5 is similar to the At/IND/1-2 tablets, in that the results confirmed the presence of phosphorus, mapping data showed this to be in the same areas as calcium, indicating the use of dicalcium phosphate (Figure 6.24). It is also clear that there is no indication of a coating present on the tablet, as the excipients are present throughout the cross section.

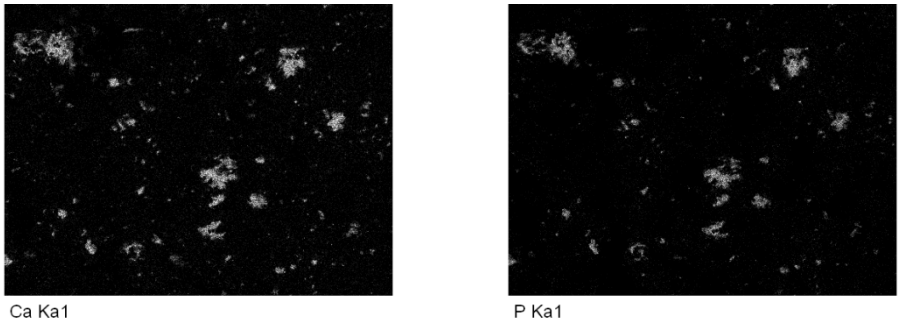

Figure 6.24: Elemental maps of Calcium and Phosphorous for At/IND/5

Maps for the At/IND/4 - 10 tablets indicate distinct regions of calcium, silicon and magnesium in the tablet core.

The EDX data for At/IND/11 – 13 is summarised in Table 6.13. Results indicate that At/IND/11 is similar in formulation to At/IND/1 - 2. At/IND/12 & 13 are of a similar elemental composition and the quantification data is comparable for the two tablets. At/IND/12 & 13 are similar in composition to At/IND/11, however like with the samples of At/IND/4, 6 - 10; there is an absence of phosphorous on the surface of the cross section.

Table 6.13: Comparison of EDX quantification data for At/IND/11 – 13 tablets

Element	**Concentration (%)**		
	At/IND/11	**At/IND/12**	**AT/IND/13**
Carbon	52.21	53.90	53.53
Nitrogen	7.86	13.71	12.42
Oxygen	37.39	31.49	32.71
Sodium	0.13		0.08
Magnesium	0.33	0.13	0.27
Aluminium		0.04	
Silicon	0.59	0.35	0.61
Phosphorous	0.74		
Chlorine	0.04	0.03	0.03
Calcium	0.73	0.35	0.35

Mapping data for AT/IND/11 confirms the presence of the excipient dicalcium phosphate, as the elemental maps for calcium and phosphorous show identical distribution. Calcium and phosphorous were absent in the At/IND/12 and At/IND/13 samples.

The EDX results for the AT/IND/14 - 16, show the same elemental composition (Table 6.14); however, there is variation within the quantification data. There are differences in the elemental composition for these samples compared to other Indian atenolol tablets, interestingly calcium, phosphorous and chlorine are absent.

Table 6.14: Comparison of Quantification data At/IND/14 – 16 tablets.

Element	**Concentration (%)**		
	At/IND/14	**At/IND/15**	**At/IND/16**
Carbon	58.80	62.08	62.30
Nitrogen	4.68	5.80	6.73
Oxygen	35.03	31.78	30.08
Sodium	0.11	0.11	0.13
Magnesium	0.25	0.08	0.11
Silicon	1.13	0.15	0.64

The data shows variation in the concentration of all elements identified except for sodium. Elemental mapping shows that sodium is more evenly distributed in At/IND/14 and At/IND/15, than that of At/IND/16 (Figure 6.25).

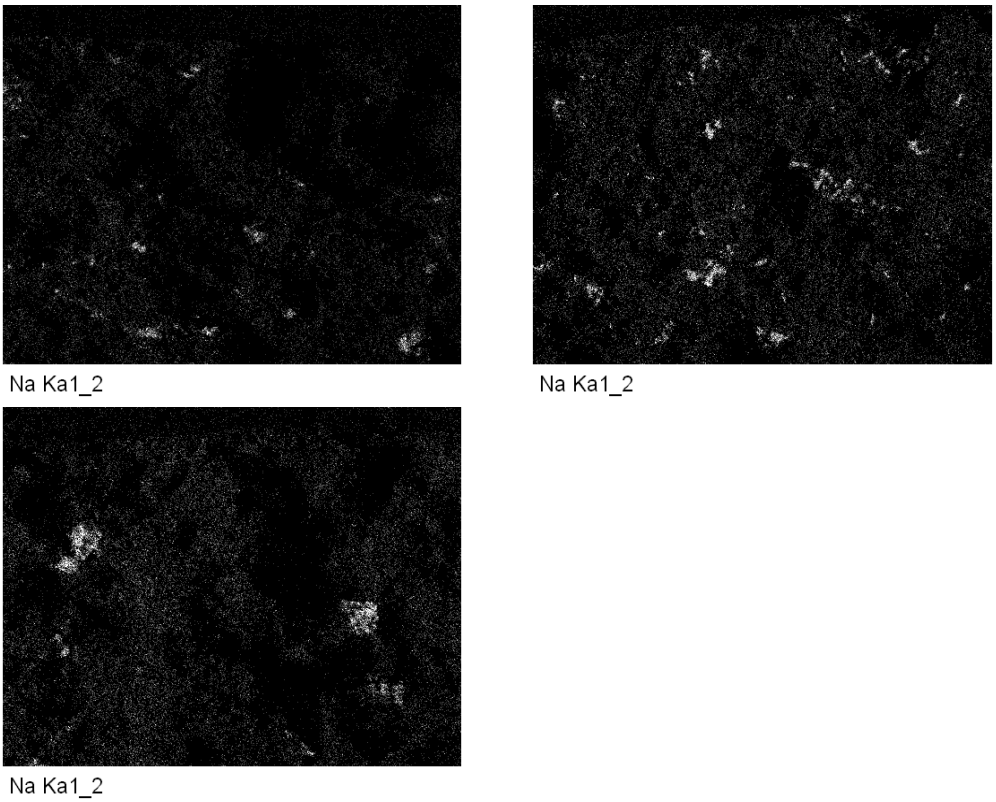

Figure 6.25: Elemental maps of Sodium for A) At/IND/14, B) At/IND/15 and C) At/IND/16

Similar applies for silicon and magnesium, At/IND/14 has a higher concentration present and is distributed across the tablet; this is also the same for At/IND/16, however At/IND/15 contains a small distinct region.

The EDX results for At/IND/18 indicates similar ratio of carbon, nitrogen and oxygen to other atenolol formulations from the same region. Mapping indicates the presence of magnesium and silicon in the same positions.

6.4.1.3 *Pakistani Tablets*

The elemental composition of the At/PAK/1 tablet contains carbon, nitrogen and oxygen at similar ratios to other atenolol tablets. There is an absence of calcium, though this is probably due to formulation differences. The quantification data is summarised in Table 6.15 and compared to another tablet (At/PAK/2) from Pakistan. Mapping data indicates that magnesium and silicon are widely distributed and are in identical positions. There is a clear region of sodium, indicating the possible use of sodium croscarmellose, as it is a single large particle. The At/PAK/2 tablet is similar in formulation to At/PAK/1, in that carbon, nitrogen and oxygen are present in similar quantities. The formulation differs in the absence of sodium and silicon. Mapping indicates that magnesium is concentrated on one side of the tablet. This may be due to the homogeneity of the sample, or limitation of the technique. However, it is unlikely to be technique related as the rest of the sample was mapped successfully.

Table 6.15: Comparison of EDX quantification data for At/PAK1 and At/PAK/2 tablets

Element	**Concentration (%)**	
	At/PAK/1	**At/PAK/2**
Carbon	60.27	63.28
Nitrogen	8.63	7.01
Oxygen	29.77	29.67
Sodium	0.24	
Magnesium	0.34	0.03
Silicon	0.56	
Chlorine	0.19	0.02

6.4.1.4 *Saudi Arabian Tablets*

The EDX data for the Saudi Arabian tablets is summarised in Table 6.16.

Table 6.16: Comparison of EDX quantification data for Saudi Arabian tablets

Element	Concentration (%)				
	At/SA/1	**At/SA/2**	**At/SA/3**	**At/SA/4**	**At/SA/5**
Carbon	61.81	59.33	62.62	62.96	58.22
Oxygen	35.97	38.28	36.53	36.57	37.47
Sodium	0.08	0.07	0.44	0.23	0.28
Magnesium	2.10	2.28	0.08		1.19
Aluminium			0.03		0.46
Silicon			0.19	0.15	1.53
Chlorine			0.08	0.09	0.15
Titanium	0.04	0.04	0.03		0.69

The EDX results for the At/SA/1 and At/SA/2 are comparable, as they contain the same elemental composition and at similar concentrations. The elemental composition is similar to that of the UK tablet (At/UK/7) from the same manufacturer, however the Saudi Arabian tablets contain sodium and the quantification results indicate a variation in the atomic percentage. Mapping data indicates that these tablets are coated with titanium dioxide.

The EDX results for At/SA/3, At/SA/4 and At/SA/5 indicate a difference in elemental composition, At/SA/3 and At/SA/5 are identical in the elemental composition identified, and however these are not at similar concentrations. The elemental composition of At/SA/4 shows the absence of magnesium, aluminium and titanium, this may be due to formulation differences or the area examined.

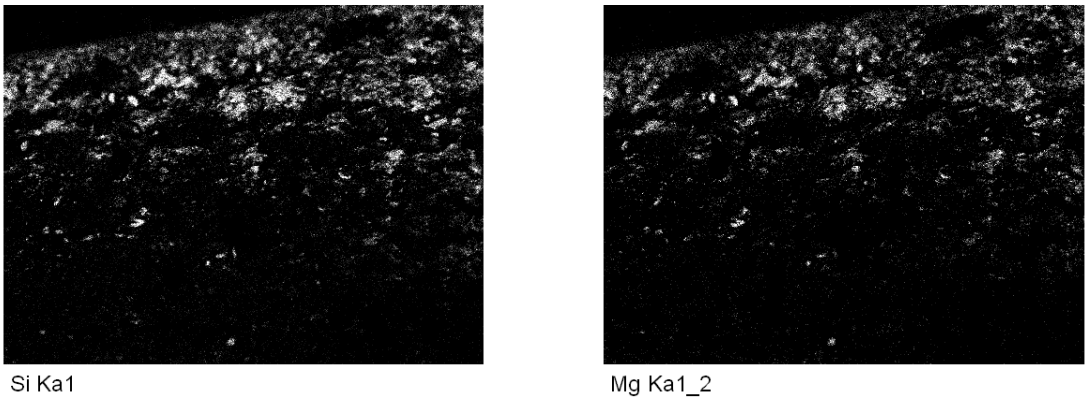

Figure 6.26: Elemental maps of silicon and magnesium for At/SA/5

Mapping indicates that there is no distinct coating present in these tablets. The map for At/SA/5 shows magnesium and silicon present in the same positions; however, they are concentrated closer to the edge of the tablet unlike in any other formulation (Figure 6.26). The level of titanium is significantly higher in the At/SA/5 sample; mapping data indicates that the titanium is present in the coating.

6.4.1.5 *Nepalese Tablets*

The EDX data for the Nepalese samples is summarised in Table 6.17.

Table 6.17: Comparison of EDX quantification data for the Nepalese samples

Element	Concentration (%)		
	At/NEP/1	At/NEP/2	At/NEP/3
Carbon	48.47	61.34	61.75
Nitrogen	4.09	7.23	7.49
Oxygen	43.45	30.79	30.20
Sodium	0.10	0.12	0.10
Magnesium	3.89	0.19	0.18
Silicon		0.28	0.23
Chlorine		0.06	0.04

The EDX results for At/NEP/2 and At/NEP/3, show identical elemental composition and good agreement between the atomic percentage quantification results. Mapping indicates similar distribution of elements between the two tablets.

The results for At/NEP/1 show a similar elemental composition to other samples manufactured by the same company (At/UK/7, At/SA/1 and At/SA/2). However, it is not identical, the quantification results differ to the other samples, the carbon and oxygen ratio differs, and the presence of nitrogen alters this ratio. There is also a higher concentration of magnesium. Mapping indicates magnesium is distributed across the tablet; however, there appears to be an absence of any distinct coating.

6.4.2 Metformin hydrochloride

6.4.2.1 *UK Tablets*

The EDX data for the different UK metformin hydrochloride tablets summarised in Table 6.18. From the quantification data, it can be inferred that the different dosages of metformin hydrochloride tablets are of a similar elemental composition, with the exception of calcium in the Met/UK/1 tablet. This may be due to differences in formulation. The quantification results show good agreement for the elements present in the two tablet samples.

Table 6.18: Comparison of Quantification data for UK Metformin Hydrochloride tablets.

Element	Concentration (%)	
	Met/UK/1	**Met/UK/2**
Carbon	50.08	49.25
Nitrogen	36.96	37.96
Oxygen	8.06	7.91
Sodium	0.08	0.08
Magnesium	0.07	0.06
Silicon	0.35	0.24
Chlorine	4.32	4.44
Calcium	0.03	
Titanium	0.06	0.06

Mapping indicates a high concentration of chlorine, which is widely distributed across the tablet; however, there are some regions with an absence of chlorine (Figure 6.27). Results also indicate the presence of titanium in a fine layer in the tablet coating, also there is an amount of magnesium present in the coating (Figure 6.28).

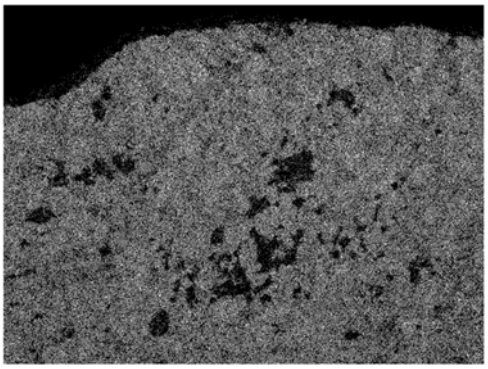

Figure 6.27: Elemental map of Chlorine for Met/UK/2

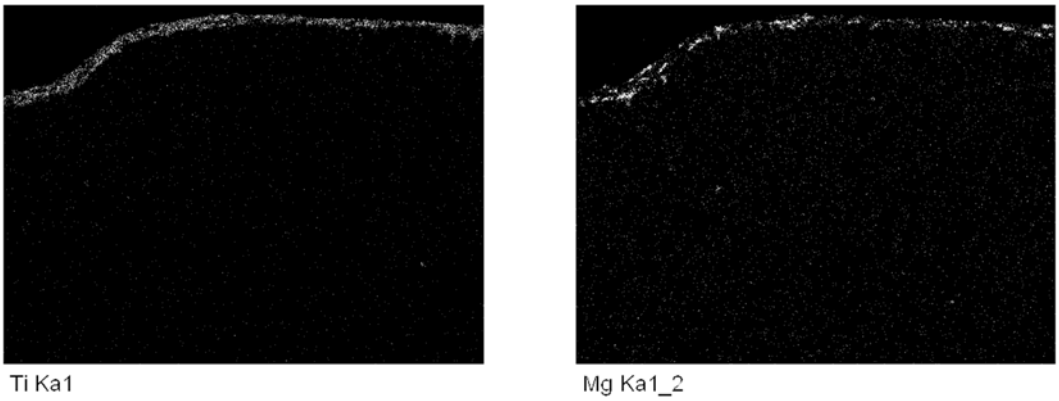

Figure 6.28: Elemental map of Titanium and Magnesium for Met/UK/2

6.4.2.2 *Indian Tablets*

The EDX data for the Met/IND/1 – 5 tablets can be summarised in Table 6.19.

Table 6.19: Comparison of EDX quantification data for Met/IND/1 -5 tablets

Element	**Concentration (%)**				
	Met/IND/1	**Met/IND/2**	**Met/IND/3**	**Met/IND/4**	**Met/IND/5**
Carbon	44.86	44.87	60.98	50.35	58.20
Nitrogen	40.31	40.66	24.58	36.71	27.97
Oxygen	9.86	9.65	10.61	7.90	10.10
Sodium				0.08	
Magnesium	0.06	0.06	0.06	0.07	
Silicon	0.28	0.25	0.16	0.26	0.14
Chlorine	4.60	4.47	3.53	4.56	3.53
Calcium	0.03	0.03			
Titanium			0.09	0.07	0.06

In general, the EDX data indicates similarities in the elemental composition between formulations and dosage for Met/IND/1 – 5 tablets. There is a marked difference between the standard and sustained release formulations; the concentration of carbon and oxygen is higher and the nitrogen and chlorine concentration is lower in the sustained release tablets. From the mapping data, chlorine is widely distributed throughout the tablet for all of the Met/IND/1 – 5 formulations. There is a clear layer of titanium coating for the Met/IND/3 – 5 tablets, possibly indicating a difference in formulation to the Met/IND/1 and Met/IND/2 tablets. Quantification data for Met/IND/6 - 8 can be summarised in Table 6.20.

Table 6.20: Comparison of EDX quantification data for Met/IND/6 - 8 tablets

Element	Concentration (%)		
	Met/IND/6	**Met/IND/7**	**Met/IND/8**
Carbon	54.17	46.90	46.87
Nitrogen	20.96	34.69	34.08
Oxygen	19.92	14.16	14.70
Sodium			0.11
Magnesium		0.09	
Silicon	0.14	0.28	0.37
Chlorine	11.88	3.87	3.87

The Met/IND/6 sample tablet contained less elements /excipients than the other tablet formulations. The chlorine concentration is considerably higher than that of other tablets. Mapping shows small distinct areas of silicon and that chlorine is widespread throughout the tablet, but again in distinct regions.

The Met/IND/7 and Met/IND/8 tablets are similar in composition and the quantification data is in good agreement, with slight differences in magnesium and silicon concentrations.

The EDX data for the Met/IND/9 and Met/IND/10 can be summarised in Table 6.21. From the quantification data, it can be observed that the different dosages of metformin hydrochloride tablets are of a similar elemental composition, with the exception of calcium in the Met/IND/10 sustained release tablet. This may be due to differences in formulation.

Table 6.21: Comparison of Quantification data for Met/IND/9 and Met/IND/10 tablets

Element	Concentration (%)	
	Met/IND/9	**Met/IND/10**
Carbon	44.38	55.45
Nitrogen	42.17	24.02
Oxygen	8.84	17.49
Sodium	0.10	0.20
Silicon	0.09	0.13
Chlorine	4.43	2.69
Calcium		0.02

The quantification results show differences in the atomic percentage of the two tablets, it is clear that the sustained release tablet is of a different formulation, whilst being of a similar composition. The data for Met/IND/10 is different to the sustained release formulations of another manufacturer (Met/IND/4 & 5) suggesting a different formulation. Mapping shows clear distinct regions of sodium that relates to large particles, suggesting the use of sodium croscarmellose as a disintegrant.

The EDX data for the Met/IND/11 and Met/IND/14 – 16 can be summarised in Table 6.22. From the quantification data, it can be inferred that the different brands of metformin hydrochloride tablets are of a similar elemental composition, with the exception of the Met/IND/16 tablet and the presence of calcium in the Met/IND/15 tablet. This may be due to differences in formulation; however, this is consistent with other sustained release tablets. The Met/IND/16 sample identified fewer elements, suggesting that very few if any excipients are included in the tablet, indicating a different formulation; SEM images also indicated a difference in morphology for this particular tablet.

Table 6.22: Comparison of EDX quantification data for Met/IND/11. Met/IND/14 – 16 tablets

Element	**Concentration (%)**			
	Met/IND/11	**Met/IND/14**	**Met/IND/15**	**Met/IND/16**
Carbon	58.32	59.28	57.54	62.76
Nitrogen	21.56	22.06	22.42	18.82
Oxygen	17.21	15.23	16.86	14.37
Silicon	0.09	0.18	0.24	
Chlorine	2.82	3.25	2.91	4.06
Calcium			0.02	

Mapping data indicates that chlorine is widely distributed across the tablets. One noticeable difference was observed for the Met/IND/15 tablet. Chlorine is distributed across the tablet; however, where there was an absence of chlorine, sodium was present (Figure 6.29).

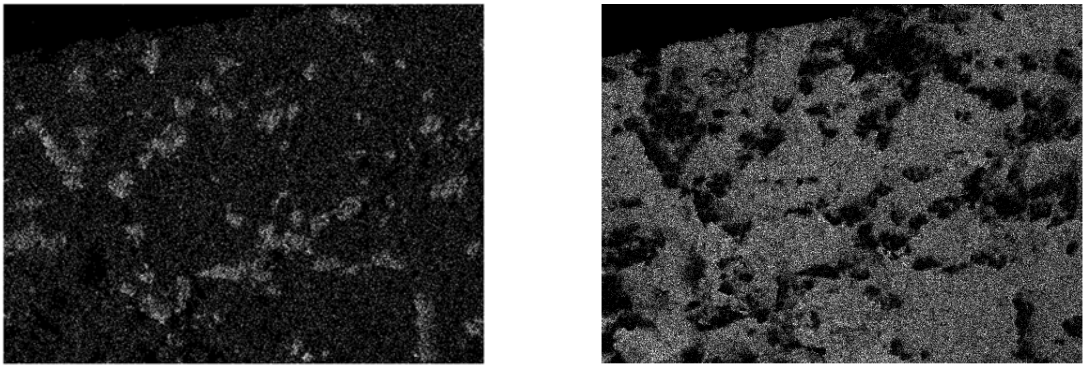

Figure 6.29: Elemental maps of Sodium and Chlorine for Met/IND/15 tablet.

The EDX data for the Met/IND/12 & 13 tablets can be summarised in Table 6.23. From the quantification data, the different formulations of Met/IND/12 & 13 tablets are of a similar elemental composition. The quantification data differs, suggesting a different formulation.

Table 6.23: Comparison of Quantification data for Met/IND/12 & 13 tablets

Element	**Concentration (%)**	
	Met/IND/12	**Met/IND/13**
Carbon	46.46	56.86
Nitrogen	39.02	21.25
Oxygen	10.24	18.19
Sodium	0.10	0.08
Silicon	0.26	0.66
Chlorine	3.90	2.97
Calcium	0.03	

Mapping data indicates a clear region of silicon in the Met/IND/13 map.

6.4.2.3 *Saudi Arabian Tablets*

The quantification data for Met/SA/1 tablet is summarised in Table 6.24. The elemental composition is similar to that of the Met/IND/16 tablet from India, although the concentration element differs significantly. The EDX data indicates the possible absence of excipients in this formulation. Mapping indicates the distribution of carbon to be closer to the tablet coating than the core.

Table 6.24: Quantification data for Met/SA/1 tablet

Element	Concentration (%)
Carbon	50.89
Nitrogen	38.77
Oxygen	6.47
Chlorine	3.57

6.5 Summary

The quantification of the API in single tablets was assessed by UV Spectroscopy and ATR FTIR Spectroscopy. The value measured by UV spectroscopy was taken as the analytical reference.

The majority of the tablets analysed (atenolol and metformin hydrochloride) by UV Spectroscopy had API levels within either the EMA (90-110%) or the FDA (80-125%) acceptable ranges of the stated dose. The exceptions were the atenolol sample At/PAK/1 and the metformin hydrochloride samples Met/IND/7 and Met/IND/13, this could indicate substandard tablets.

The same samples were analysed by ATR FTIR Spectroscopy, overall the quantification results for atenolol was low for the majority of tablets from the different countries. This may be due to interactions between the excipients and the chosen peak range used to quantify the atenolol, or it may stem from poor mixing and agglomeration issues. However, the metformin hydrochloride samples gave high quantification results; again, similar inferences concerning mixing can be drawn. SEM results for atenolol showed signs of agglomeration and non-uniform particle size for metformin hydrochloride.

Raman Spectroscopy was unsuccessful in producing reproducible quantification data for an API within a tablet; further work is required to investigate this further.

Elemental analysis performed by EDX Spectroscopy, showed differences in the inorganic content of the tablet. Particular differences were observed for the samples At/UK/7, At/SA/1, At/SA/2 and At/NEP/1; the blister packaging indicated these samples from the same manufacturer. The UK and Saudi Arabian samples were comparable to each other; however, the Nepalese sample contained a higher concentration of magnesium.

7 Comparison of Techniques

This chapter is devoted to the discussion and comparison of results presented in the previous two chapters. Results for the various techniques will now be compared against one another, whilst considering the need to provide a rapid screening method to identify SF medicines in LIC and LMIC's.

The Raman, ATR FTIR and Mass Spectrometric techniques used in this study were all able to detect the target API's provided the level in the tablet was greater than 1% w/w.

7.1 ATR FTIR and Raman Spectroscopy

When comparing the two techniques, Raman spectroscopy offers a non-destructive technique, which can analyse whole tablet formulations, whereas ATR FTIR is unable to analyse the whole tablet and requires the tablet sample to be crushed. Both techniques are complementary and can be used in a tandem, for example some formulations can provide different Raman and IR spectra due to some bonds being Raman active and IR inactive and vice versa (Schlűcker *et al*., 2003). The ability of the two techniques to identify different bonds is important if a molecule contains polar bonds for example an OH group, which is a strong IR absorber ATR FTIR spectra can become swamped by this signal, however as it is a weak Raman scatterer the sample can be successfully analysed by Raman.

One limitation to Raman spectroscopy is the effect of fluorescence on the spectra, the fluorescence signal can overload and swamp the relatively weak Raman signal. This means that some coated samples and some API's can give a misleading spectrum. Sometimes the effect of fluorescence can be advantageous; Ricci *et al.,* (2008) used this to distinguish falsified artesunate tablets. The API artesunic acid fluoresced, enabling the group to determine that tablets that give this signal, the API was absent from the sample. The effect of fluorescence can be mitigated by changing the excitation laser wavelength, the closer the wavelength is to the NIR region the lesser the effect (Horiba, 2017).

The principal differences between the ATR FTIR and Raman spectroscopy can be summarised as:

i) Raman is able to identify APIs in a whole tablet formulation whereas ATR FTIR cannot gain sufficient surface contact to give good spectra.
ii) ATR FTIR has a fixed penetration depth, whereas the depth penetration in Raman depends on the surface materials.

iii) ATR FTIR can more readily provide quantitative data than Raman.
iv) The Raman spectra generally have fewer peaks than IR.
v) Raman spectra can be prone to the effect of fluorescence.
vi) It is difficult to deal with mixtures due to the complex spectra produced. Generally, handheld Raman spectrometers failed for mixtures.

It is also important to consider the ability to quantify the level of API in a single tablet. ATR FTIR spectra are easy to quantify using the OPUS software once a calibration plot has been generated. The ability to do this within the analysis software allows for rapid quantification of a single tablet. However, quantification of an API using Raman spectroscopy is not as simple as the method for ATR FTIR. Complex models are computed using multivariant analysis (PLS or PCA), these models are generated using large data sets on multiple batches of/and tablet repeats. Often the data used for multivariant analysis has been acquired using Surface Enhanced Raman spectroscopy (SERS).

One example of the differences between techniques is for the API atenolol and the tablets At/IND/1 - 2 and At/IND/5 - 10. The ATR FTIR spectra suggests that the tablets are not identical in formulation, as the spectra are inconsistent with one another (Figure 7.1), with additional components between 3500 and 3800cm^{-1} and around 1000cm^{-1}.

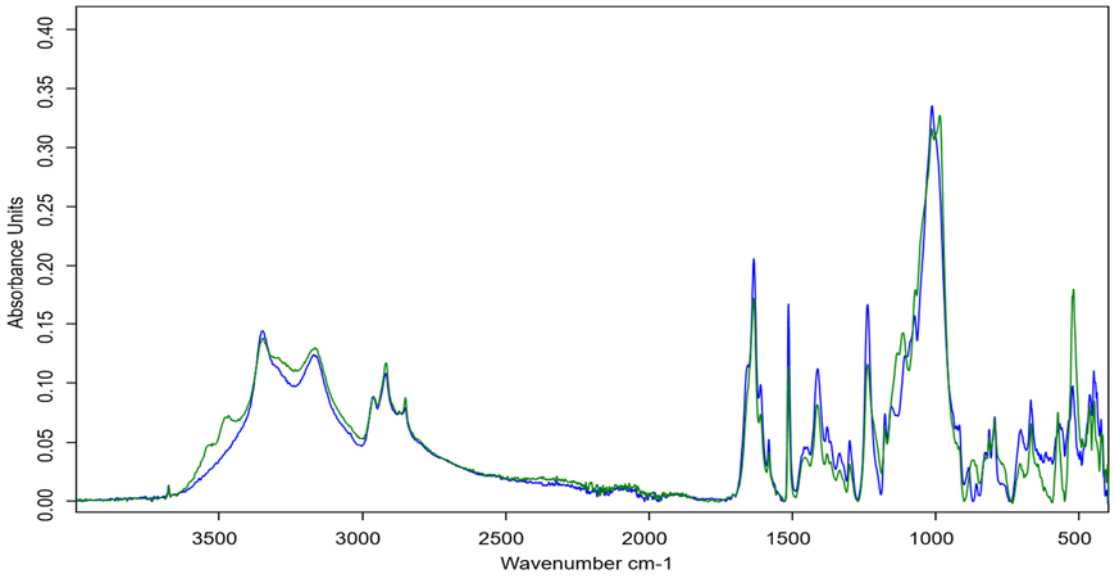

Figure 7.1: ATR FTIR spectra of At/IND/1 (Green) and At/IND/5 (Blue

This is surprising, as the samples are all from the same manufacturer, the only difference being that At/IND/1 – 2 contain a lower dosage of API (25mg) compared to the AT/IND/5 – 10 samples (50mg).

The Raman spectra for the same samples indicates a difference between the 25mg and 50mg tablets (Figure 7.2). Using the excipient library created for this research, it is possible to infer that the difference in spectra was as a result of the presence of dicalcium phosphate (988cm^{-1}).

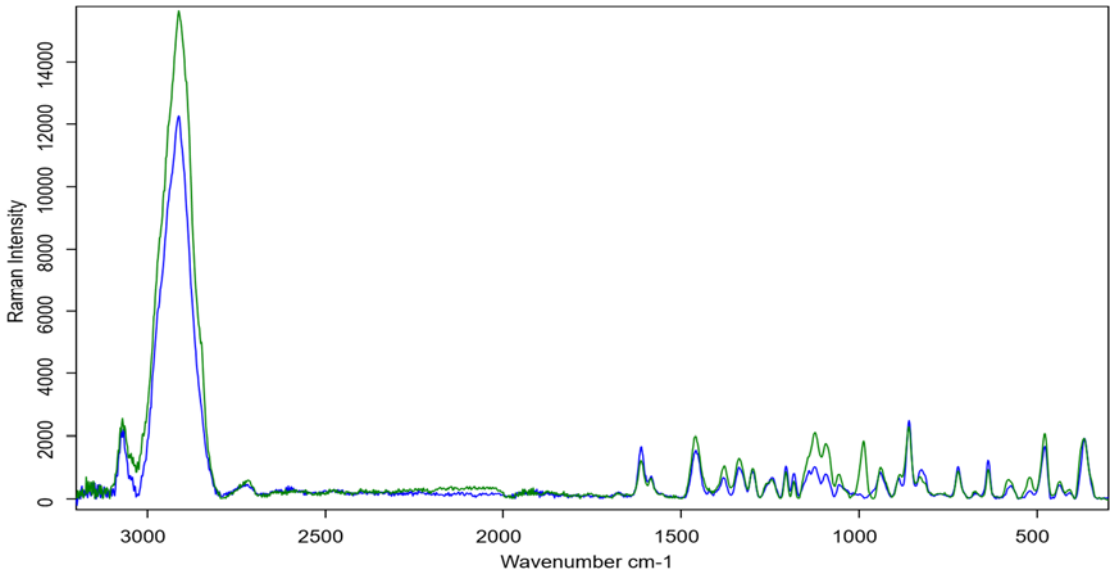

Figure 7.2: Raman spectra of At/IND/1 (Green) and At/IND/5 (Blue)

Figure 7.3A and B compares the ATR FTIR and Raman spectra for At/IND/1 and At/IND/5. For the purpose of this comparison, the Raman spectra have been plotted on the same x-axis as the ATR FTIR spectra. Both the Raman and ATR FTIR data have been normalised using minimum and maximum normalisation.

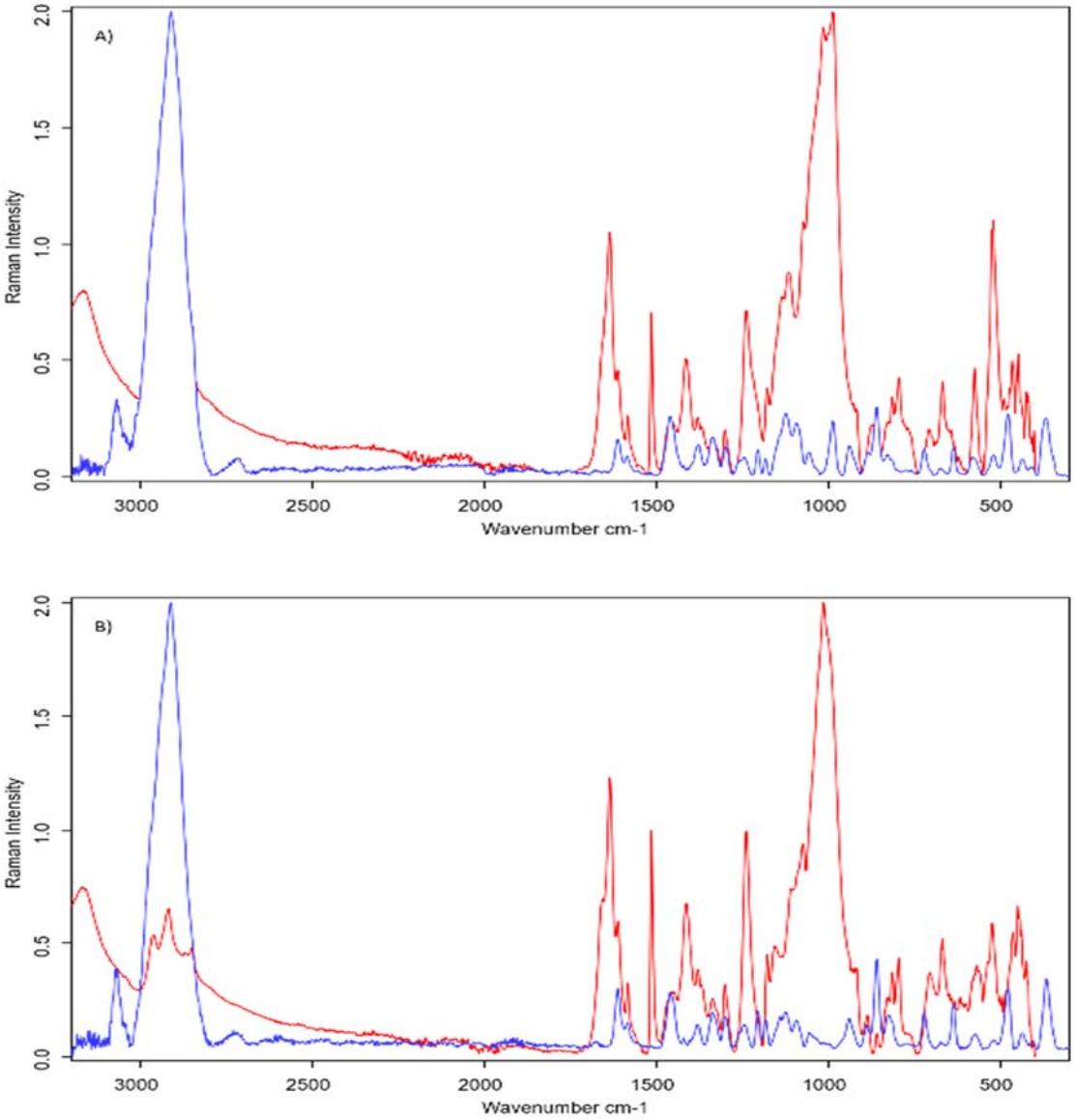

Figure 7.3: A) Raman spectra of At/IND/1 (Blue) and ATR FTIR spectra of At/IND/1 (Red), B) Raman spectra of At/IND/5 (Blue) and ATR FTIR spectra of At/IND/5 (Red)

Whilst the pre-normalised Raman spectra were more intense, differences can be observed between the two types of spectra for the samples. One of the characteristic peaks for atenolol (1515cm^{-1}, aromatic) is present in the ATR FTIR spectrum, but is absent in the Raman spectra. There are other notable differences in the spectra highlighting the differences between IR active and Raman inactive bonds.

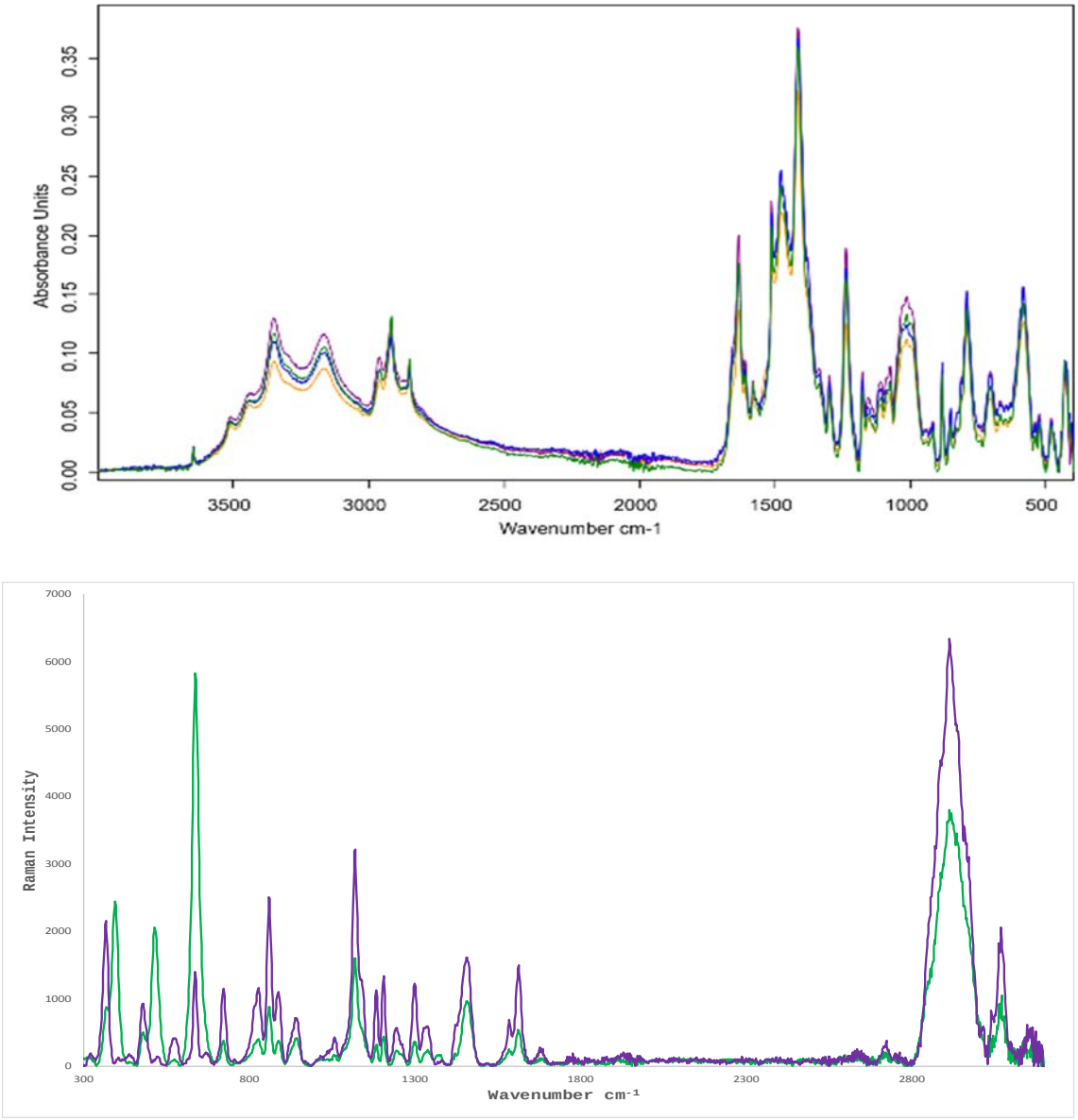

Figure 7.4: A) ATR FTIR spectra of At/SA/1 (Green), At/SA/2 (Blue), At/UK/7 (Purple) and At/NEP/1 (Yellow), B) Raman spectra of At/SA/1 (Green), and At/NEP/1 (Purple)

The spectra obtained for the atenolol sample At/NEP/1 differs for the two techniques when compared to other samples from the same manufacturer. Figure 7.4 shows the ATR FTIR and Raman spectra for At/NEP/1 compared to comparable samples. The ATR FTIR spectra is identical to one another, whereas the Raman spectra show differences between At/NEP/1 and the other samples.

The Raman spectra shows peaks at different positions (368cm^{-1} and 480cm^{-1}) and an additional peak at 576cm^{-1}. This may indicate the possibility of titanium dioxide in the coating or the presence of a polymorph exhibiting similar lattice structure with different bonding. Analysis of

the sample by X-ray Diffraction (XRD) would be able to confirm the presence/ absence of a polymorph. Variation in crystal structure may give rise to the additional peak at 576cm^{-1}. This is a good example of the benefit of the sharp peaks for titanium dioxide in the Raman spectra, which can be clearly observed, versus the broad IR peaks, which can be masked by other excipients.

Another example of the differences between Raman and ATR FTIR spectra is the antimalarial sample AM/ZIM/4. Whole tablets from the AM/ZIM/4 sample were analysed, multiple tablets were analysed with varying success. Both techniques could identify differences in peak intensity and shape for the samples, however the peaks were harder to distinguish in the ATR FTIR spectra (Figure 7.5A), this is due to ATR FTIR being a surface technique (Bugay and Brush, 2001 and Planinšek *et al.*, 2016) and the limited depth of sample penetration. The spectra for the crushed sample shows more information and the peaks are well defined, with no distinguishable peaks for titanium dioxide.

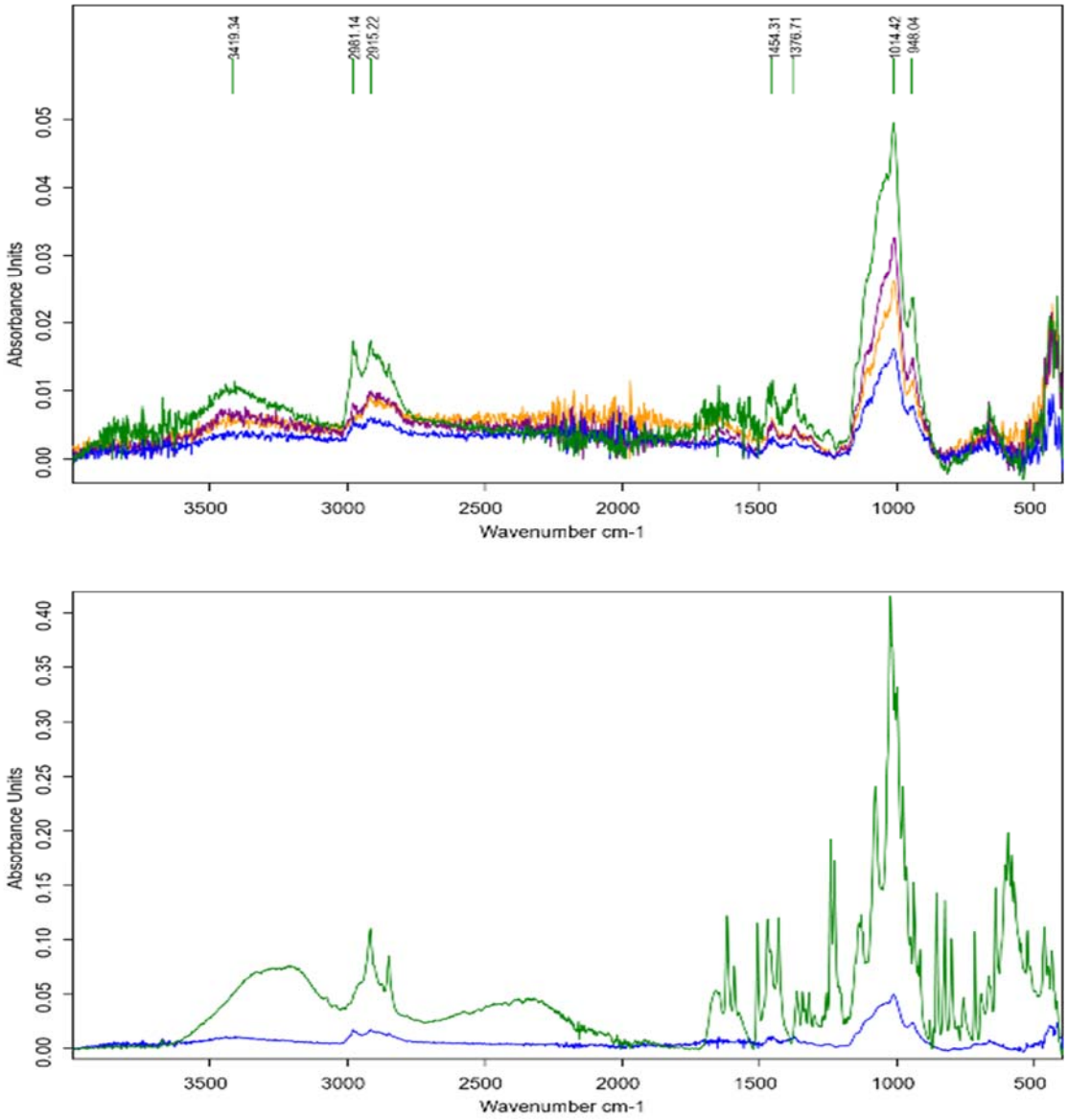

Figure 7.5: A) ATR FTIR spectra of AM/ZIM/4 tablet 1 (Green), tablet 2 (Blue), tablet 3 (Purple) and tablet 4 (Yellow), B) ATR FTIR spectra of AM/ZIM/4 whole (Blue) and crushed (Green)

The Raman spectra for AM/ZIM/4 showed differences between the tablets, particularly tablet 1 (Green) and 5 (Red) (Figure 7.6). The ratio of peaks for the excipient titanium dioxide and the API quinine sulphate differ between the two samples.

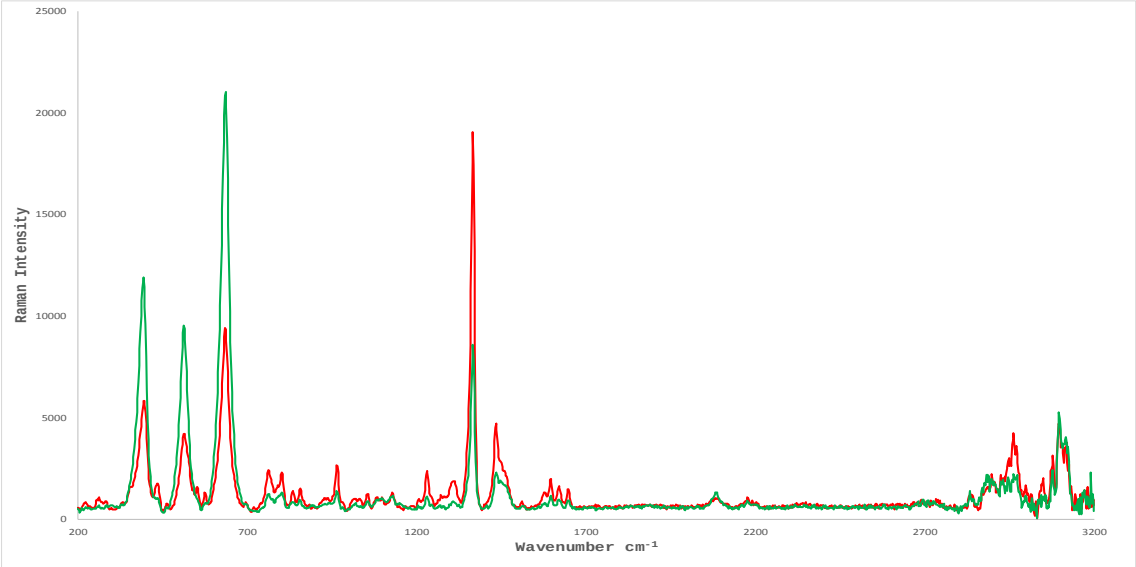

Figure 7.6: Raman spectra of AM/ZIM/4 tablet 1 (Green), and tablet 5 (Red)

The spectrum for tablet 1 suggests a higher concentration of titanium dioxide, whereas the spectrum for tablet 5 infers a higher concentration of API within the tablet. These differences may be attributed to the surface coating and distribution of API and excipients within the tablet, or may indicate the possibility of a difference in the level of API within the tablet. Further analysis by SEM/EDX would enable the amount of titanium in the samples to be quantified.

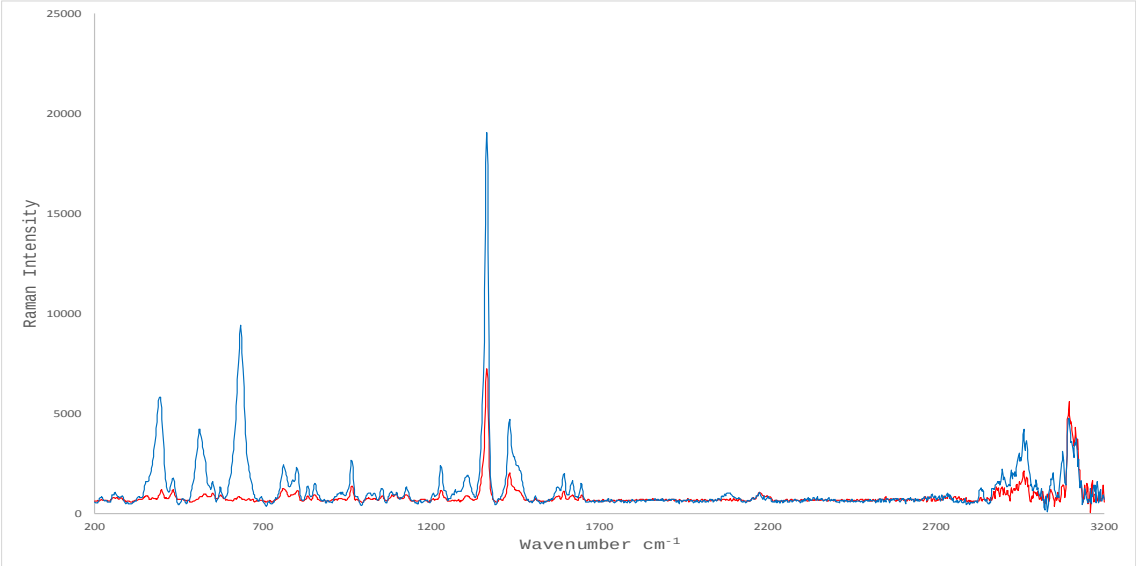

Figure 7.7: Raman Spectra for AM/ZIM/4 whole tablet (Blue) and powdered (Red) using the FORAM-2 spectrometer

Clear differences between the powdered and whole tablets (Figure 7.7) were observed for the tablet samples AM/ZIM/4. The spectra for the whole tablet were at a stronger peak intensity and

showed some peaks at a slightly different wavenumber and others absent. The peaks at 394cm^{-1}, 518cm^{-1} and 638cm^{-1} for the whole tablet can be attributed to titanium dioxide; this may be due to the coating of the tablet. The peaks at 1362cm^{-1} and 1430cm^{-1} for the whole and powdered tablets are characteristic of quinine sulphate.

This data for the AM/ZIM/4 quinine sulphate tablets are an example of the limitation of Raman spectroscopy. The samples are from the same identical batch, yet the Raman spectra (Figure 7.6 and Figure 7.7) indicate differences in the nature of the sample. Peaks observed in the whole tablet spectra indicate the presence of titanium dioxide, the intensity of these peaks vary amongst the batch of tablets. This variation indicates a difference in the thickness of the coating layer, suggesting poor QC during manufacture. The spectrum for the powdered sample confirms that titanium dioxide is contained within the coating layer of the tablet, as the peaks for it are absent. The intensity of the quinine sulphate peaks also varies within the batch. Modern Raman instruments use PCA techniques to minimise these effects in order to identify the samples as being the same.

7.1.1 Raman Instrument Comparisons

This research compared the application of handheld and benchtop Raman spectrometers to identify a target API within a tablet. This was met with varying levels of success. The three different spectrometers used to analyse atenolol, were all able to identify the API using an analytical reference standard. However, the MIRA-3 handheld spectrometer struggled to obtain a spectrum for the tablet samples, both whole and crushed; this was due to the effect of fluorescence. The other handheld spectrometer (BRAVO) was able to compensate this effect due to the dual wavelength capability and therefore could generate a meaningful spectrum. A study by Assi, (2015a) confirmed the outcomes in this research regarding fluorescence and dual wavelength spectrometers. The group were able to mitigate the effect of fluorescence for antimalarials by using a dual wavelength spectrometer, as was found in this study by comparing the MIRA-3 and BRAVO spectrometers to identify atenolol.

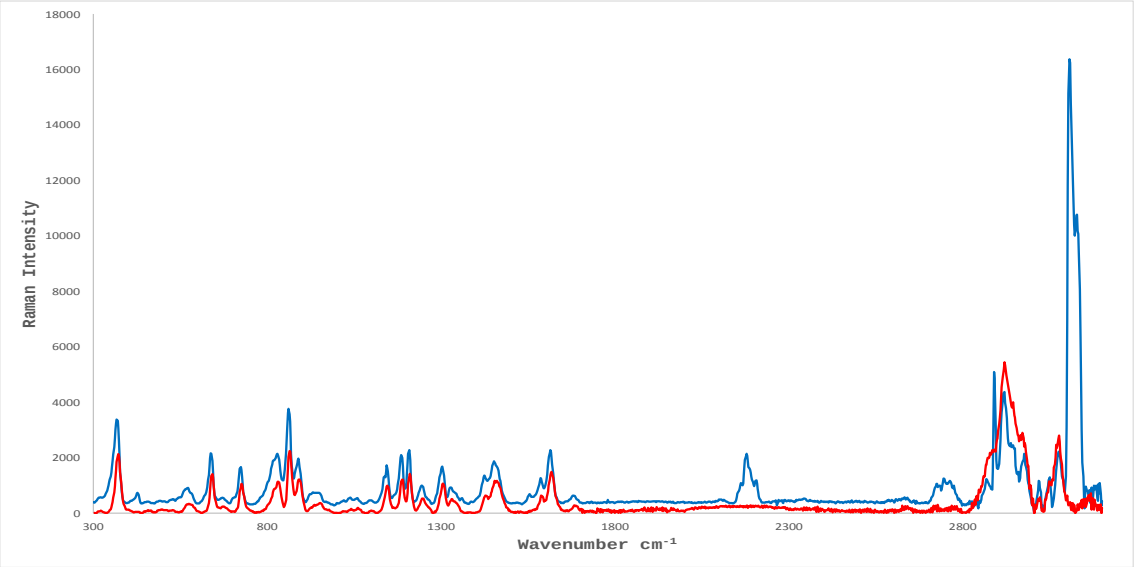

Figure 7.8: Raman spectra of atenolol (analytical standard) using BRAVO handheld spectrometer (Red) and FORAM-2 spectrometer (Blue)

The spectra obtained for the benchtop system (FORAM-2) showed small differences when compared to the handheld spectrometer (BRAVO) (Figure 7.8). Peaks at 2180cm^{-1}, 2744cm^{-1}, 2890cm^{-1} and 3106cm^{-1}, could be observed using the FORAM-2 spectrometer but are absent in the BRAVO spectrum. The spectra for the handheld and benchtop spectrometers were comparable to one another with the FORAM-2 identifying the extra peaks for the API atenolol. Differences between handheld and benchtop spectrometers has been observed by other groups (Bugay and Brush, 2010), whilst the advent of the handheld spectrometer takes 'the laboratory to the sample' (Assi *et al.*, 2015b), the handheld spectra can appear noisy as the signal to noise ratio is poor and may lead to false negative results (Dégardin *et al.*, 2017).

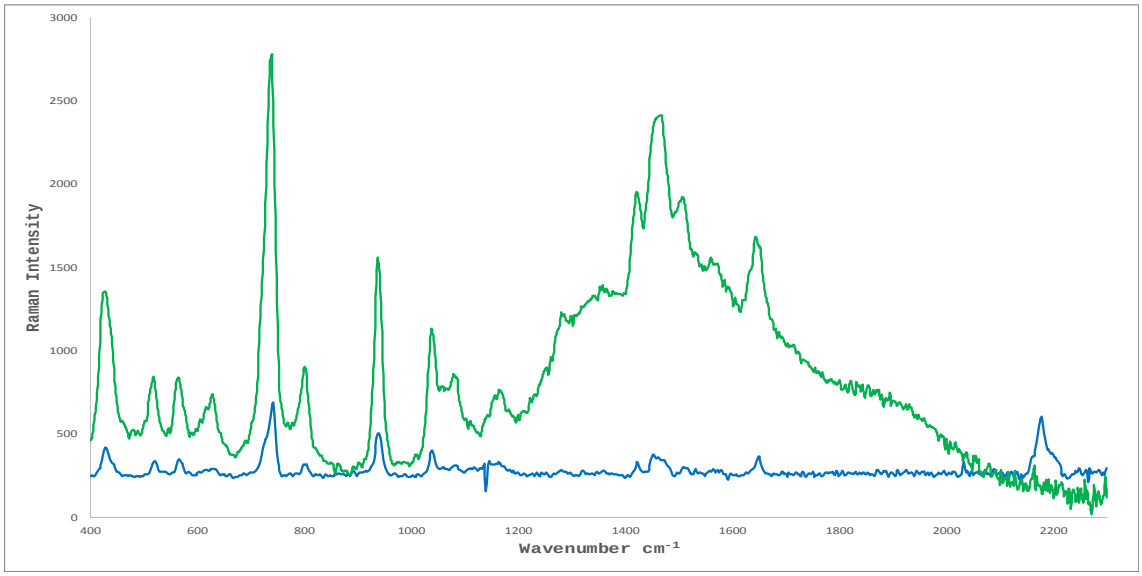

Figure 7.9: Raman spectra of metformin hydrochloride (analytical standard) using FORAM-2 spectrometer (Blue) and MIRA-3 handheld spectrometer (Green)

The API metformin hydrochloride was analysed using two different spectrometers (MIRA-3 and FORAM-2). Whilst both spectrometers could identify the API, clear differences could be observed between the spectra, notably peak intensity and the effect of fluorescence. As with atenolol, the MIRA-3 was prone to this effect when analysing metformin hydrochloride, however unlike with atenolol the peaks were well resolved and defined (Figure 7.9).

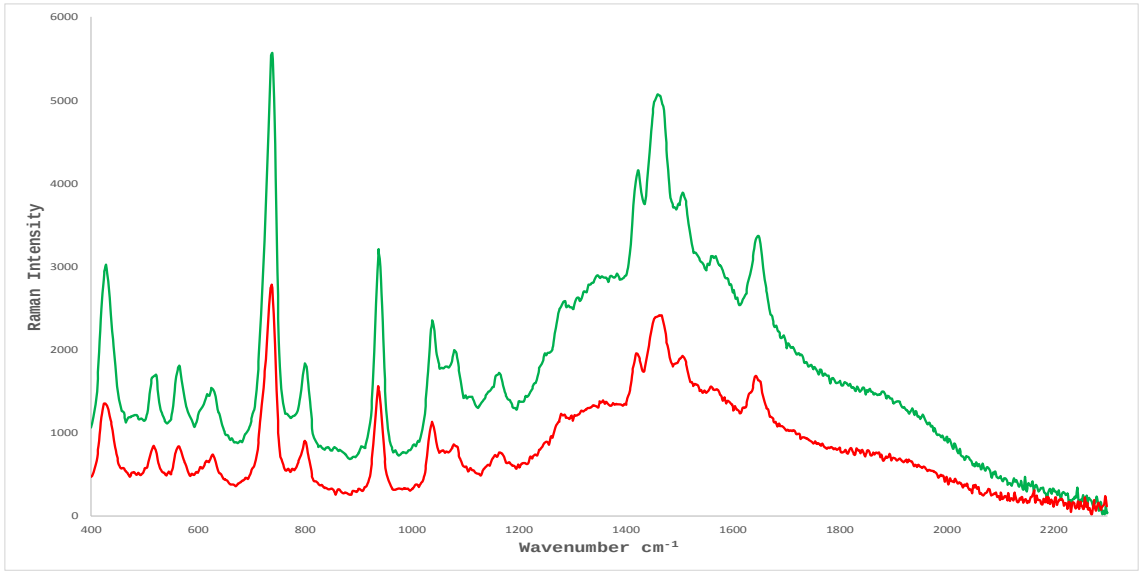

Figure 7.10: Raman spectra of Met/IND/13 (Green) and metformin hydrochloride analytical standard (Red) using MIRA-3 handheld spectrometer

Tablets that contained multiple API's, for example Met/IND/13, which contained 5mg of glipizide and 500mg metformin hydrochloride, showed no difference between the sample and analytical reference. The glibenclamide was unable to be detected by Raman spectroscopy (Figure 7.10); this is because the API glibenclamide is at a low concentration within the tablet (~2%) as opposed to ~80% of the API metformin hydrochloride. As a result of the metformin hydrochloride tablets containing a high percentage of API, there are no obvious excipient peaks in the spectrum.

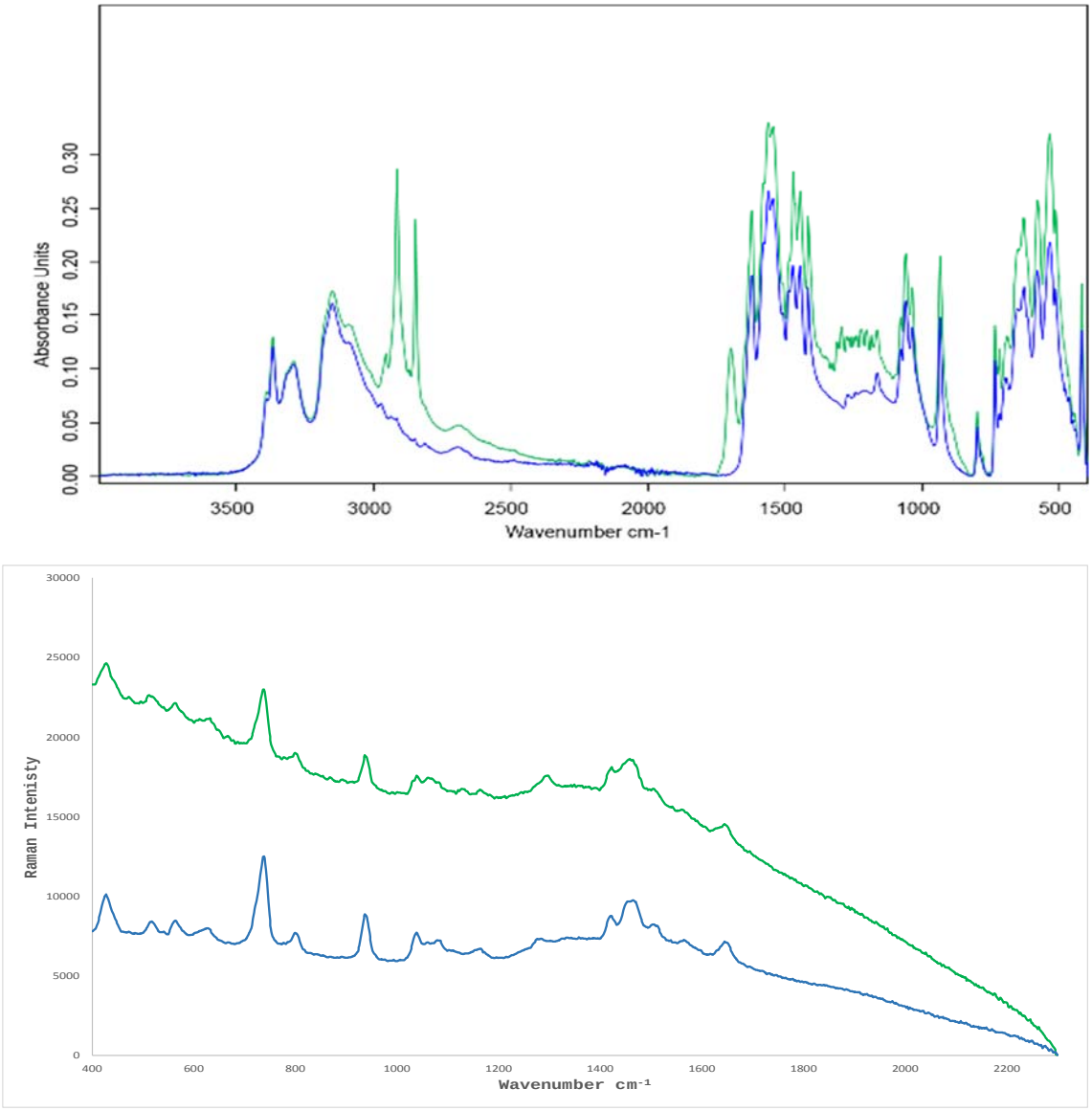

Figure 7.11: A) ATR FTIR spectra of Met/IND/2 (Blue) and Met/IND/4 (Green), B) Raman spectra of Met/IND/2 (Blue) and Met/IND/4 (Green) using MIRA-3 handheld spectrometer

Differences in spectra were observed for standard and extended/ sustained release tablets using ATR FTR, this is due to differences in formulation (Figure 7.11A). The ATR FTIR spectra showed differences between the standard release (Met/IND/2) and the extended/ sustained release (Met/IND/4) (Figure 7.11A); extra peaks are due to the additional compound being IR active, whereas the Raman spectra (Figure 7.11B) showed little or no difference, suggesting the addition of Raman inactive material.

Both techniques (Raman and ATR FTIR) an are capable of single tablet analysis, which is important in this research.

7.2 UV and ATR FTIR Spectroscopy

UV analysis of an API is an established methodology that is used by the BP and other pharmacopoeias to assay various APIs to ensure quality control specifications are met. Analysis by UV often resorts in a large quantity of tablets being used for analysis, for example, the BP 2017 requires 20 tablets of atenolol for the assay. Whilst in HIC and QC laboratories this does not present a problem, in LIC and LMIC the reality is a patient/ healthcare provider does not have this volume to analyse. They often only have a small amount and rarely can be spared; the majority of tablets obtained from India for this study were sold in 'fortnightly' 14-day blister packs. Another issue with the UV assay test is that if there were variation within a batch, this would not be detected, as the overall result is an average of the 20 tablets. Single tablet analysis allows the quantification of the API of a single dose, this is important, as a patient is unable to normalise the level of API, as is done by batch quantification. The individual would be susceptible to fluctuations in the level of API and may not receive the correct dosage.

UV analysis requires solvent extraction of the API and can often be time consuming. The proposed rapid methodology of quantification of an API by ATR FTIR is a novel technique for the application of atenolol and metformin hydrochloride. It offers 'single tablet' capability and analyses both API and excipients, as opposed to just the API as with UV analysis.

UV analysis responded as expected for all of the tablets analysed for both atenolol and metformin hydrochloride. Using and adapting the methodology outlined in the BP 2017, it was possible to quantify the level of API in a single tablet for atenolol and metformin hydrochloride.

The quantification results by UV analysis for atenolol tablets ranged from 74% to 122% of percentage API content. The majority of samples fell within the acceptable limits as outlined by the EMA (90% – 110%) and FDA (80% to 125%).

When comparing the UV quantification results with the ATR FTIR quantification results, several observations can be made. The UV measurements were all trended to the upper limit set by the BP or above this limit for percentage content of atenolol, whilst there is a general trend of the ATR FTIR data towards the lower value of API content. Figure 7.12 compares the quantification data obtained for both UV and ATR FTIR analysis. The data for the Indian samples At/IND/1, At/IND/2 and At/IND/11, show a concentration effect, as the results analogues to the Beer Lambert Law, additional work is required to investigate this further.

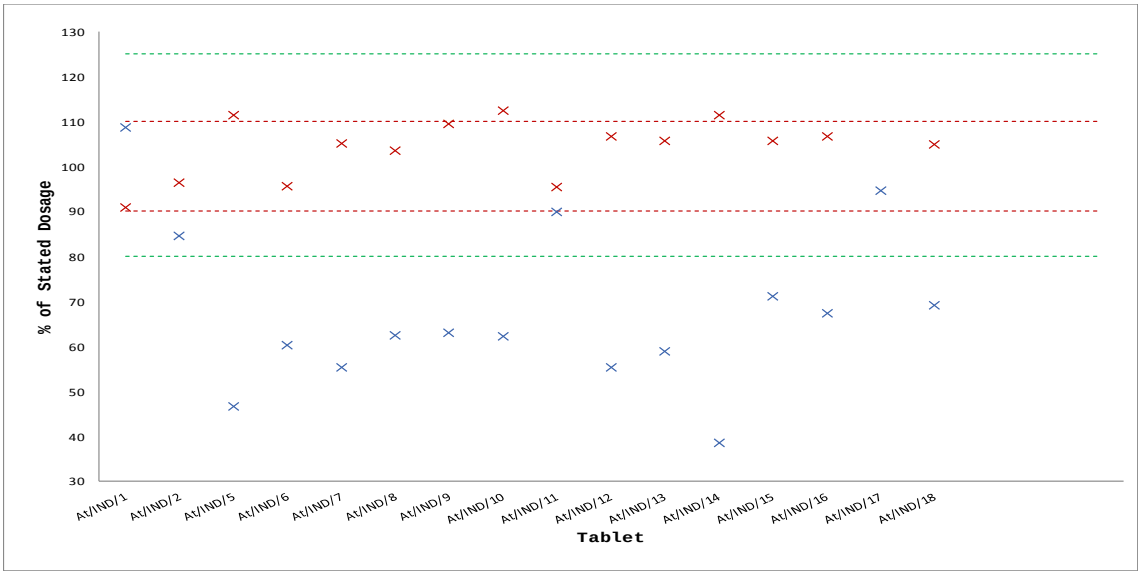

Figure 7.12: Percentage of stated dosage of Indian atenolol tablets as calculated by UV (X) and ATR FTIR (X) with EMA (--) and FDA (--) limits

Whilst both methods identified substandard tablets, the API content as calculated by ATR FTIR was generally lower. One factor that may attribute to these values being lower is a limitation of this technique; samples need to be crushed and sufficiently mixed/homogenised, Particle size and homogeneity is critical and agglomeration of particles can affect this. Salari and Young (1998) reported that not attaining a 'perfect mixture' affects the quantification of powders. It is possible that the atenolol agglomerated and thus caused issues with the calibration plots, resulting in low quantification values. It is important to ensure that the crushed sample is mixed and homogenous sufficiently, in order to allow for uniformity of particle size.

The quantification data for metformin hydrochloride, as analysed by UV analysis ranged from 78% to 137%, with the bulk of the samples falling within the acceptable limits as outlined by the EMA (90% to 110%) and FDA (80% to 125%). Those that fell outside of the range were generally lower with the exception of Met/IND/7, which was significantly higher than expected. This may be due to the overall quality of the tablet sample, upon receipt of the sample several tablets were

broken and appeared to be of poor quality within the blister packaging. In contrast to the atenolol results, the metformin hydrochloride samples analysed by ATR FTIR trended towards the upper limit (105%) and above.

This again may be due to poor mixing and /or choice of excipient for the calibration mixtures; metformin hydrochloride tablets contain a large percentage content of API, in this study the percentage content of API ranged from 70% to 95% of the total tablet weight. Metformin hydrochloride tablets can be classed as containing a high percentage concentration of API; because of this, there are no obvious excipient peaks in the ATR FTIR spectra, as discussed in the previous section. Since the percentage API content within the tablet is so high and few excipients are used in the formulation, selection of the calibration excipient is important. Maize starch was selected for this study as it is present in the highest concentration within a tablet, however due to the API concentration being considerably higher, this may have influenced the calibration plot particularly at high excipient concentrations, resulting in a low R^2 value (0.8915) and poor linearity. However, this methodology does have potential as shown by Lawson *et al.*, (2014a); it can be applied to the quantification of other API's, in particular paracetamol, which occurs at comparable levels (85%) in tablets. The technique does have the potential for the quantification of atenolol and metformin hydrochloride, further work is required to investigate optimum mixing and excipient interactions. Whilst ATR FTIR provides a rapid screening methodology for quantification and identification of a target API within a tablet, it is important to cross-reference any new method against an established one to ensure its viability.

Identification of atenolol and metformin hydrochloride reference material by ATR FTIR and Raman in the presence of excipients was possible at >5% w/w and is viable within 5 minutes, as opposed to 30 to 60 minutes for UV analysis. Quantification of the target API can be obtained from the same sample within the same analysis time.

7.3 Mass spectrometry

Results from DIP MS showed promise and the technique can be used to identify the components of mixtures of APIs in a sample. This technique enabled compound specific data to be obtained. The target API's in the antimalarial samples analysed for this study were identified by DIP MS. The spectral data was generally quite simple, often with only two or three data points being produced per API identified. Further work is required to develop this as a novel technique for the application of identifying SF medicines. One significant limitation to this study was the reliability of the equipment. A consequence of the DIP, is that it is directly inserted into the MS source; contamination of the source from excess sample or carry over is a major concern. The lack and

availability of parts (in this case the ion volume) and specialist knowledge to remedy any issues hindered the throughput of samples and availability of this technique.

7.4 Elemental Analysis

Elemental analysis by EDX is limited, as it is only a surface technique and can be time consuming to section and mount the tablet samples. A significant limitation is the inability to detect lighter elements; though newer, more expensive detectors are able to detect boron and lithium.

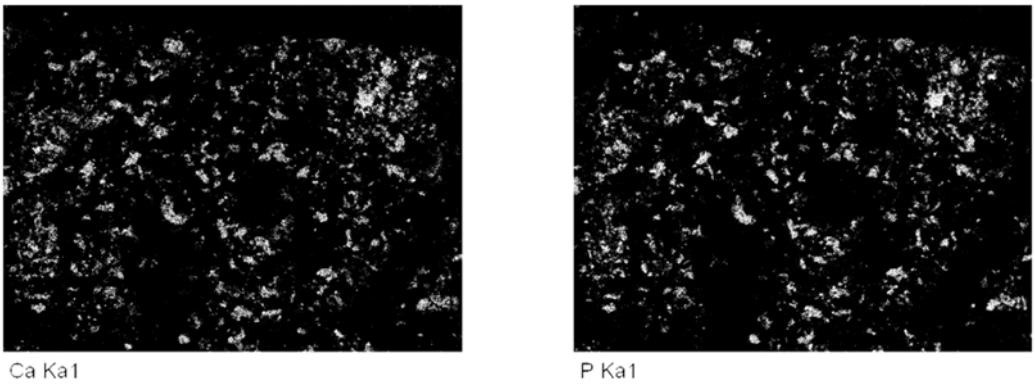

Figure 7.13: Elemental maps of Calcium and Phosphorous for At/IND/1

However, this technique is successfully able to deformulate a tablet, by mapping the spatial resolution of the elemental composition and quantifying the data. By combining the mapping and the quantification data, it is possible to infer the excipient composition of some formulations. An example of this is the samples At/IND/1 and At/IND/2. Raman spectroscopy identified the presence of the excipient dicalcium phosphate; EDX mapping data was able to confirm the presence of this excipient in the sample cross section (Figure 7.13).

Whilst there is an absence in published literature of the application of SEM/EDX to identify SF medicines, substantial work is required to validate this novel technique as worthwhile. Though EDX analysis cannot determine the chemical composition and spatial distribution like Raman imaging systems, it can identify the elemental composition and is able to quantify this data.

7.5 Discussion

Table 7.1 summarises the various analytical methods used in this research. The majority of these methods can be classed as rapid, with UV, DIP MS and SEM/EDX requiring a longer analysis time. ATR FTIR spectroscopy was able to both identify and quantify the API in a crushed a tablet, whereas Raman spectroscopy was able to identify the API in both a whole tablet and powdered sample. However, Raman spectroscopy was unable in this research to quantify the amount of API. Both types of MS analysis investigated in this research was able to identify the API in a powdered sample. Whereas SEM/EDX did not identify the API, however it was able to give the spatial distribution and quantification of individual elements.

Table 7.1: Comparison of various analytical methods with respect to tablet sample analysis

Analytical Method		**Sample**	**Technique**	**Sampling Depth**	**Detection Capability**	**Analysis Time**
UV	Quantification	Extract API in solution	Fixed Wavelength	Bulk	Able to quantify the amount of API in a tablet to 0.1% of tablet weight	>30 minutes
ATR FTIR	Identification	Powder	Fingerprint	Surface	Identified all of the target API's	~2 minutes
	Quantification	Powder	Peak Area	Surface	Detection limits are 5 % for atenolol & 10% for metformin hydrochloride	< 5 minutes
Raman	Identification	Tablet/ Powder	Fingerprint	Sample dependent	Identified all of the target API's and some excipients	~2 minutes
	Quantification	Powder	Peak Height	Sample dependent	Calibration data gave poor linearity. Further work is required.	< 5 minutes
DIP MS	Identification	Powder	m/z value	Bulk	Identified atenolol and the antimalarials chloroquine phosphate, quinine sulphate, lumefantrine, further work to alter the scan range would enable artemether, sulphadoxine and pyrimethamine to be detected.	10 – 15 minutes
ASAP MS	Identification	Powder	m/z value	Bulk	Identified atenolol, sulphadoxine and pyrimethamine.	~ 5 minutes
SEM/EDX	Elemental data	Tablet		Surface	Elemental spatial distribution over the surface of a cross sectioned tablet	~15 minutes
	Elemental quantification	Tablet	Elemental	Surface	Surface quantification of the above	~25 minutes

7.6 Application of this research to LIC and LMIC

Whilst it is important to consider what information the individual/ combined techniques give the analyst; significant consideration should be given to the application if these techniques to LIC's and LMIC's. Several factors need to be considered before any methodology could be implemented, these are:

- Cost
- Time
- Infrastructure
- Specialist knowledge
- Additional materials.

The issue of the cost of the equipment is a significant factor in being able to apply any new methodology to identify SF medicines. Table 7.2 shows the estimated cost for the equipment used in this study and for the equivalent handheld equipment; the costs shown exclude consumables and training.

Table 7.2: Estimated cost of equipment in the UK

Technique	**Cost (Excluding VAT) (£)**		
	Lab Based	**Benchtop (Portable)**	**Handheld**
UV		10,000 – 15,000	
ATR FTIR		15,000 – 20,000	
Raman		~70,000	35,000 – 65,000
Raman Microscopy		160,000	
SEM/EDX	150,000 +	75,000 – 100,000	
GCMS with DIP	50,000 +	50,000	

The cost of equipment varies significantly ranging from the 'relatively' cheap UV spectrometers to the expensive SEM and Raman microscopes. The handheld Raman and benchtop ATR FTIR spectrometers are priced somewhere in the middle. The cost of any equipment used to detect SF medicines in LIC and LMIC would consume a large amount of capital, and all aspects would need to be considered before any purchase. The running costs and available infrastructure to support the equipment is another factor.

Some countries in Africa do not have the infrastructure to support large pieces of equipment that would require a constant source of power, even in standby mode. Höllein *et al*., (2016) cited concerns with the lack of infrastructure in some African countries when setting up an analytical laboratory. In reality, not all LIC and LMIC have the infrastructure for a full-scale analytical laboratory, so ideally 'portable' and handheld equipment need to be considered. This would rule out a lab based SEM and a GCMS equipped with DIP, as these require a constant source of power

to ensure the system is continually kept under vacuum. A visit to the Pharmacy and Poisons Board in Kenya in 2017 by Tanna and Lawson indicated that laboratories were in fact available but with neither staff nor equipment.

The issue of usage of solvents in a technique and the requirement of other consumables is important. The cost of solvents, availability, grade (analytical, ultra-pure) and cost of disposal should be considered. Some solvents are expensive in the UK and can sometimes be in short supply, for example acetonitrile in 2009. The availability and grade of solvents in LIC and LMIC cannot be guaranteed, for this reasoning UV analysis can be discounted as a technique to detect SF medicines in LIC and LMIC.

The availability of expertise and specialist knowledge should be considered. Adequately trained personnel may not be available and as a result of this it is important that any technique implemented can essentially be 'pass or fail'. ATR FTIR and Raman spectroscopy can be implemented as sample preparation is simple and the spectrum for any sample analysed can be cross-matched to reference samples using a preinstalled library.

8 Conclusion

Review of the results obtained from the current investigation into rapid screening methods to identify substandard and falsified medicines has enabled the following exercise to be carried out.

8.1 Identification of a Mislabelled or Possibly Falsified Pharmaceutical Product

In the UK, there are significant price differences between proprietary and 'super market own', brands of common OTC medicines. Throughout much of the world, a similar situation is common in pharmacies for medicines normally available only on prescription in the UK. Recently in Nairobi (2017), for example, atenolol tablets were purchased from a pharmacy at different costs per tablet. This situation creates the opportunity for the replacement of one product by another and also the challenge to identify that this exchange of one formulation for another has occurred.

The Raman, ATR FT/IR and EDX techniques, detailed in previous chapters, were used to identify examples of atenolol tablets mislabelled as a proprietary product.

8.1.1 Raman Analyses

67 tablet samples were analysed as detailed in Section 4.3.4 and the PCA plot derived from this data (Figure 8.1) is shown below. The yellow group on the right of the plot were a proprietary brand with 3 examples from 2 different countries within this grouping. The yellow group at the top of the diagram (AT/NEP/1) whilst packaged as the proprietary medicine were clearly different to the other samples. Within the much larger grouping in the centre of the diagram was the data obtained from examples of the same generic medicine from a range of different countries.

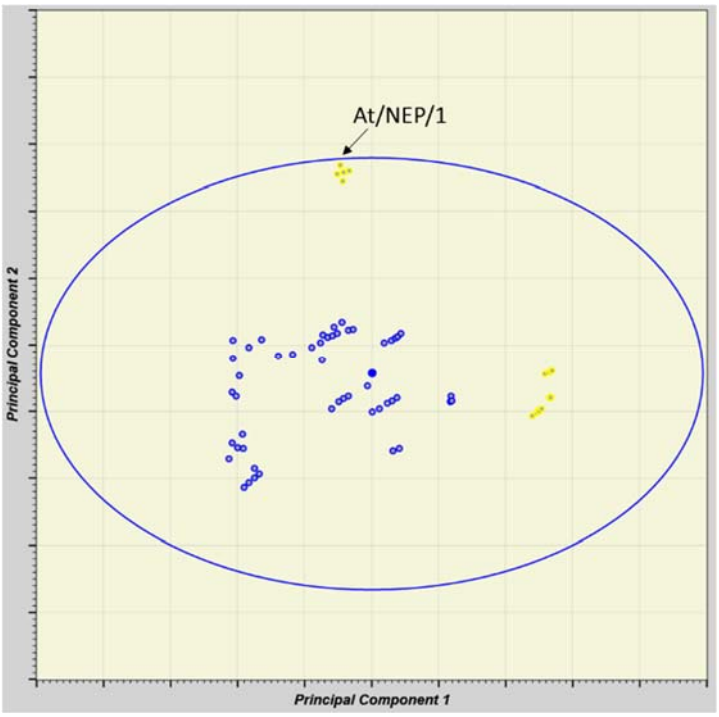

Figure 8.1: PCA Plot of the Raman spectra for whole At/UK/7 (Yellow), At/SA/1 (Yellow), At/SA/2 (Yellow), At/NEP/1 (Yellow) and other generic atenolol tablets (Blue)

Clearly, there is a difference between these two groups of supposedly the same proprietary formulation. Figure 8.2 clearly shows increased levels of titanium dioxide on the surface of the three proprietary tablet samples from the UK and Saudi Arabia.

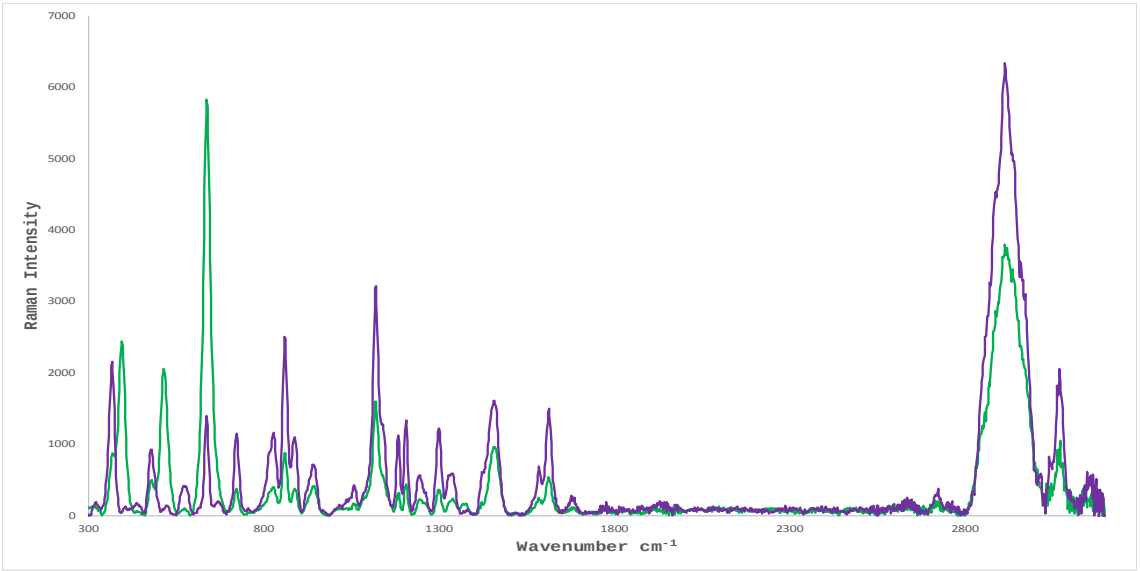

Figure 8.2: Raman Spectra of At/SA/1 (Green) and At/NEP/1 (Purple) using the BRAVO spectrometer

The Raman spectroscopy of tablet formulations is frequently affected by fluorescence, the extent of which is dependent on the nature of the formulation i.e. the excipients present in the tablet. The fluorescence spectra of the tablets can therefore be used as an indicator of the excipients used in the tablets under investigation. The data in Figure 8.3 shows that the proprietary tablet examples have low but very reproducible fluorescence spectra whereas the spectra for all the other examples exhibited a much greater intensity across the spectral range studied. The 'suspect' proprietary labelled tablets showed a fluorescence spectrum similar to but somewhat different to the spectra for the general group of generic medicines.

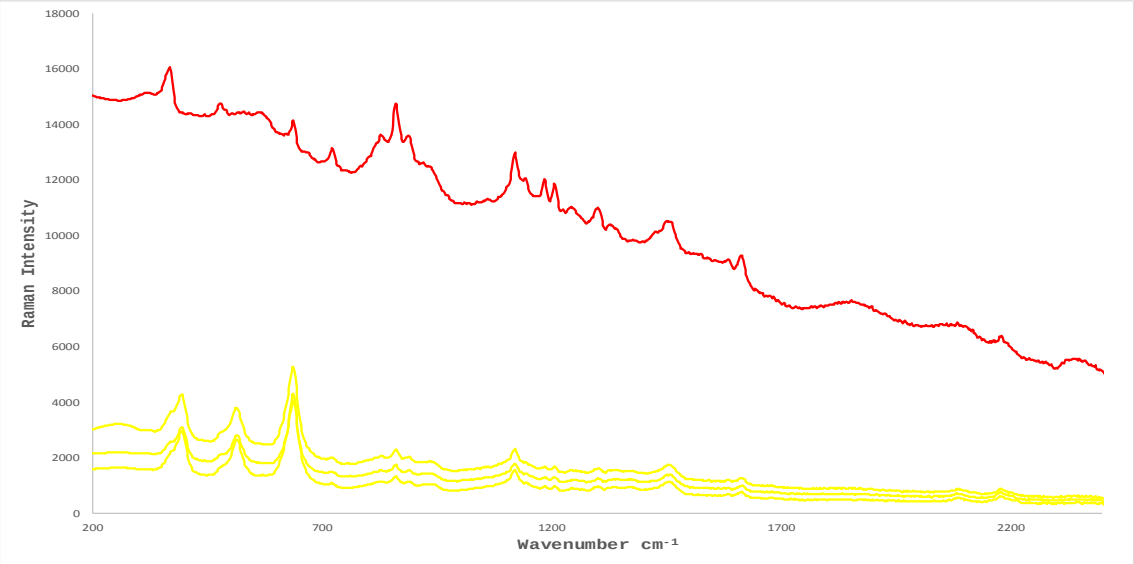

Figure 8.3: Raman Spectra of whole At/UK/7 (Yellow), At/SA/1 (Yellow), At/SA/2 (Yellow), and At/NEP/1 (Red) tablets

8.1.2 ATR-FT/IR Analyses

Comparison of the ATR spectra showed little difference between the spectra of the crushed tablet samples (Figure 8.4). This is in line with the Raman data, which is more surface sensitive.

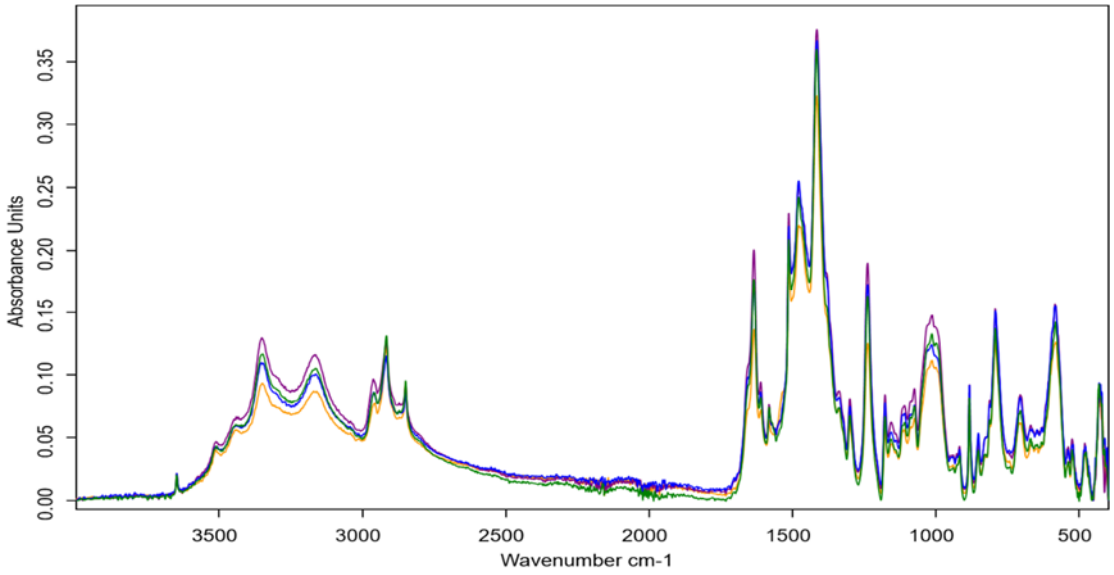

Figure 8.4: ATR FTIR Spectra of crushed At/SA/1 (Green), At/SA/2 (Blue), At/UK/7 (Purple) and At/NEP/1 (Yellow) samples

8.1.3 SEM/EDX Analyses

Elemental analysis also showed differences in the data. The results (Table 8.1) for At/NEP/1 show a similar elemental composition to other samples manufactured by the same company (At/UK/7, At/SA/1 and At/SA/2). However, it is not identical, the quantification results differ to the other samples, the carbon and oxygen ratio differs, and the presence of nitrogen alters this ratio. There is also a higher concentration of magnesium. Mapping indicates magnesium is distributed across the tablet; however, there appears to be an absence of any distinct titanium dioxide present in the coating.

Table 8.1: EDX quantification data for cross-sectioned atenolol tablets by the same manufacturer

Element	**Concentration (%)**			
	At/UK/7	**At/SA/1**	**At/SA/2**	**At/NEP/1**
Carbon	58.53	61.81	59.33	48.47
Oxygen	38.22	35.97	38.28	43.45
Nitrogen				4.09
Sodium		0.08	0.07	0.10
Magnesium	3.20	2.10	2.28	3.89
Titanium	0.05	0.04	0.04	

8.1.4 Discussion

From this study it appears that sample At/NEP/1may be either

- A mislabelled tablet,
- A falsified tablet.

A tablet in the wrong box would give a Raman PCA signal in the main grouping. Clearly, this is not the case; the sample is clearly not simply mislabelled.

DIP mass spectrometry confirmed only the target API to be present and UV/Vis analysis confirmed the expected level of API to be present.

A deliberate or accidently change to the formulation cannot be determined.

This is however, a clear indication of the values of the combined approach to the investigation of substandard and falsified medicines.

The elemental analysis for substandard and falsified medicines is a novel concept. EDX spectroscopy has not yet been applied to this area of research. Raman and ATR FTIR analyses have been used extensively to investigate counterfeits by focussing on the identification of the API. One unique focus of this work is the use of these techniques to identify a falsified ‘proprietary product’ based on the analysis of excipients.

9 Further Work

As a result of the experimental work performed and a review of the results obtained, the following areas have been identified where scope exists for future work:

- Further analysis is required to confirm if the samples identified by this research are substandard or not.

- To investigate the mixing issues presented for the quantification of an API by ATR FTIR and the selection of excipients for calibration mixtures.

- Raman spectroscopy showed promising results, to enable this as a rapid screening method for atenolol and metformin hydrochloride, further work is required to investigate quantification of an API.

- To apply ATR FTIR and Raman to other API's and determine if it is possible to quantify the API.

- To investigate the potential of SEM/EDX in deformulating a tablet and look to develop the novel technique. Also, investigate the use of cathodoluminscence in identifying SF medicines.

- All new methods need to be proven, any potential rapid techniques need to be validated against an established one. Further work to test the potential of ATR FTIR against LCMSMS.

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Appendices

Is it what it says on the packet? ATR FTIR provides a rapid answer to counterfeit tablet formulations

G Lawson, E Turay, R Armitage, L Goodyer and S Tanna

1. Introduction

How many people would question the reliability of a packet of paracetamol or even atenolol bought whilst away on holiday?

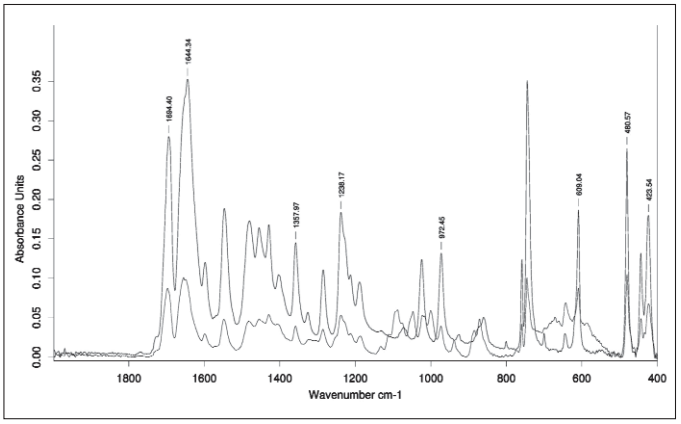
Counterfeiting of medicines is a global phenomenon that affects both developed and developing countries and especially in medicines sourced via the internet.[1] In the UK the level of counterfeit medicines is thought to be between 1 and 2% whilst elsewhere in the world figures as high as 40% are cited by the WHO. A counterfeit medicine is a medicine which is deliberately and fraudulently mislabelled in regards to identity and source which apply to both branded and generic products. Products may or may not have the correct excipients, exclusion of active pharmaceutical ingredient (API) or insufficient quantities of API or fraudulent packaging. The effects of counterfeit medicines on patients can range from the difficult to detect and quantify to an important cause of unnecessary morbidity, mortality and loss of public confidence in medicines and the health systems. In recent years more and more incidents of deaths resulting from sub-standard or counterfeit medicines have been reported. Recently in Pakistan, for example, more than 400 deaths were thought to have resulted from counterfeit cardiovascular medication. The identification of these materials causes problems for the regulatory authorities both in the Healthcare Supply Chain and also for Border Agency staff. The problem with international visitors is particularly acute for countries like Saudi Arabia, where large numbers of pilgrims often bring excess medicines with them which need to be rapidly assessed.

Rapid assessment implies a screening process rather than the time consuming in depth analyses offered by conventional laboratory equipment such as GC-MS or NMR.[2] The first step is a visual assessment of barcodes or more frequently now the use of two dimensional barcodes read by smart phones. This provides rapid communication between supplier, importer and the border agency but is however only limited to assessing the packaging rather than the contents. Chemical test kits are limited to specific active ingredients, a problem not faced by a portable Raman spectroscopy system which is being trialled in this area and can be programmed for many APIs.[3] Costs and concerns about the robustness of the system have raised some concerns.

In this paper an alternative spectroscopic system Fourier Transform Infra-Red (FTIR) spectroscopy utilising Attenuated Total Reflection (ATR) sampling[4] has been investigated. FTIR systems form the basis of compound identification in the British Pharmacopoeia (BP)[5] and the combination with the ATR sampling system removes the need for any prior solvent extraction to separate the API from the excipients. A tablet formulation would simply be finely powdered and a small sample of the API + excipients powder placed on the ATR window and the resultant spectrum produced. As can be seen from Figure 1 the spectrum obtained for caffeine from the tablet formulation is virtually indistinguishable from the spectrum obtained from the reference material.

Figure 1

Comparison of reference (red top) and tablet samples for Caffeine.



This approach therefore appears to provide a 'fingerprint method' to rapidly assess if the expected API is present in the dose format being tested. An assessment of the API dose level may also be made from the intensity of the peaks characteristic of the API of concern. This determination would be dependent on the detection capabilities of the ATR FTIR system and very low dosage levels may not be currently detectable.

2. Experimental

2.1 Instrumentation

The ATR FTIR spectra were recorded using a Bruker Alpha system equipped with a platinum diamond single reflection sampling station. Spectra of each sample were collected at a resolution of 2cm^{-1} over the range 400 – 4000cm^{-1}. Replicate spectra, a minimum of three, were collected for all calibration and trial samples. The BP based analyses were run on a Thermo Electron Helios Omega UV/Vis instrument using Vision Lite

Is it what it says on the packet? *continued*

2.2 software.

2.2 Chemicals and sample preparation
The reference samples: caffeine, atenolol and paracetamol were > 99% analytical grade, whilst the excipients used to prepare the calibration mixtures were all pharmaceutical grade. The excipients used included hydroxymethyl cellulose, titanium dioxide and magnesium stearate.

Reference spectra of the authentic APIs were produced from finely ground samples placed on the ATR sample station. The FTIR system was zeroed prior to each sample series.

For the quantitative calibration samples several single excipients were used for individual experiments since the excipients in the test samples would be unknown. Powder quantitative calibration and validation samples were prepared by mixing suitable amounts of the API and excipient to cover the normally encountered dosage range. These calibration samples are expressed as a percentage by weight of the API in the excipient. The anticipated ranges were: Paracetamol 30-80% w/w and Atenolol 10-40% w/w based on the tablets studied. After mixing using a pestle and mortar to ensure homogenisation powder samples were placed on the ATR station and replicate spectra were recorded. This process was repeated for several different samples taken from the calibration mixture to ensure reproducibility. Quantitative data was obtained from the Opus 6 software for spectral peaks characteristic of the target APIs to produce calibration data for the subsequent analyses.

Samples for the BP analyses were prepared as detailed in the British Pharmacopoeia 2010 modified for single tablet analysis.

Strips of tablets of both paracetamol and atenolol were obtained adventitiously from pharmacies in the UK, Pakistan, and India. These trial samples were identified as follows:

- Paracetamol – UK: 4 x 500mg and India: 5 x 500mg
- Atenolol – UK: 3 x 50mg, Pakistan 3 x 50mg + 1 x 100, India 2 x 50mg + 1 x 25mg

3. Results and Discussion
3.1 Identification of the API
The overlay spectra in Figures 2 and 3 comparing the spectrum of the neat API with that obtained from a typical pill formulation, show that the anticipated API can be readily identified to be present. The correlation between the spectra confirms that no other API is present at significant levels.

3.2 Determination of the dosage level
ATR measurements are affected by the degree of surface contact between the sample and the sampling diamond and a fine powder sample is therefore important for reproducible data. Furthermore, as seen by Mazurek,[3] increasing the number of samples analysed will improve the data as shown in Table 1 where the r^2 regression coefficient from different calibration diagrams demonstrates these effects.

Table 1
Methods to improve reproducibility

Mixing Time	No Spectra	r^2 Value
Short	1	0.8
Short	3	0.9
Long	3	>0.95

Figure 2
ATR-FTIR spectrum in absorbance mode of UK tablet (blue) and pure Paracetamol reference (red)

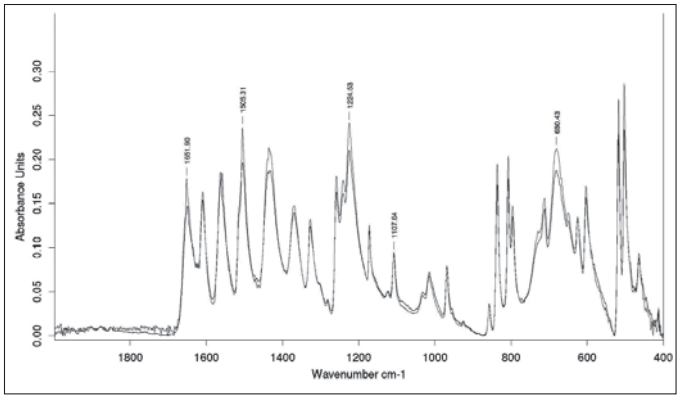
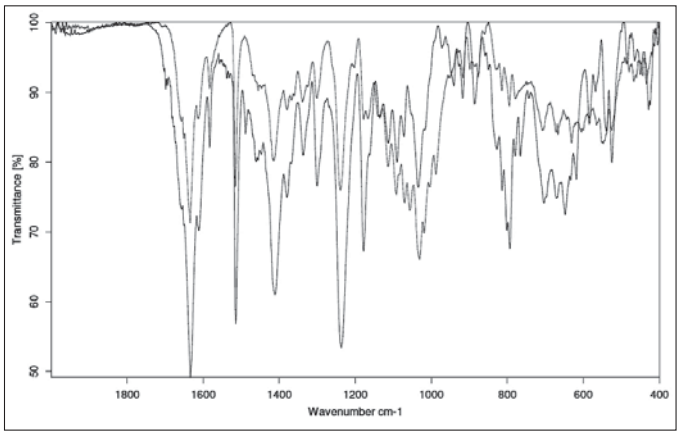


Figure 3
ATR-FTIR spectrum in transmission mode of UK tablet (red) and pure Atenolol reference (blue)



Once suitable calibration data has been obtained the percentage level in the tablet formulation can be derived and the actual dosage level calculated from the mass of the pill.

The results derived from this investigation are shown in Table 2.

Once the calibration data has been prepared the identification and quantification of the target API is

generally available within 5 minutes of opening the tablet packet. As can be seen the results for the UK tablets show good agreement. Two of the paracetamol samples from India are not as expected, one with a low dose 438mg vs 500 and more surprisingly one at 628 vs 500mg. The measured doses in all the atenolol samples from Pakistan were lower than stated on the packet and these samples were analysed using the BP method to confirm these findings. The results obtained for the Pakistan atenolol samples were #A 34mg and #B 61mg confirming the ATR data.

Table 2
Quantitative analysis results

		Paracetamol		Atenolol		
Dose		**Expected**	**Measured**		**Expected**	**Measured**
UK	#1	500	523	#A	50	45
	#2	500	490	#B	100	104
	#3	500	505	#C	100	98
	#4	500	512			
India	#1	500	438	#A	25	20
	#2	500	502	#B	50	42
	#3	500	525	#C	50	45
	#4	500	485	#D	50	30
	#5	500	628	#E	50	30
Pakistan				#A	50	40
				#B	100	58

Note all results subject to a 10% error.

3.3 Discussion

The results demonstrate the potential of the ATR FTIR approach to rapidly assess the actual components of a medicine against those stated. This approach provides a regulatory authority with the means to rapidly identify a suspect material using only bench top analytical equipment with no consumables other than electricity. Further analysis would be required to confirm the presence of either a substandard or counterfeit material.

The cardiovascular tablets were obtained from Pakistan shortly after the problems in this area were reported and the measured low levels of atenolol may be related to these incidences.

The substandard levels of paracetamol and atenolol may well be the results of poor mixing at the production stage and it is unlikely that these tablets would provide therapeutic levels of the relevant drug under the prescribed conditions.

The elevated paracetamol level found in one set of samples from India would be of concern for people prescribed 8 or more tablets a day where the total dose would be around 5024 mg rather than the 4000 expected.

This work provides further evidence regarding the poor quality of some of pharmaceuticals available in many developing countries.[6] It is quite likely that this is due to issues relating to poor quality control of the manufacturing processes and the impact on the health of the population in such countries is hard to quantify. For the traveller visiting such areas this data serves as a warning that wherever possible medicines should be purchased before departure, including items such as paracetamol used to treat minor conditions.

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References:

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Counterfeit or just poor quality control?

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INTRODUCTION

- The World Health Organisation (WHO) estimates that ~10% of medicines globally are either Substandard or Falsified (SF)[1].
- To protect the public there is a genuine need for rapid “single tablet capable” qualitative and/or quantitative analytical method.
- Counterfeiters have been known to use low amounts of active pharmaceutical ingredient (API) in their products to enable them to pass qualitative screening tests. As a result, patients receive sub therapeutic doses of the drug, leading to ineffective treatment, therapeutic failure and even drug resistance[2].
- Counterfeiters are finding ways to bypass established quality control (QC) techniques, therefore the development of new techniques is required.

RESEARCH AIMS

- The overall aim of this project is to develop Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR FTIR), as a rapid instrumental method to identify and quantify the API in tablet formulations.
- This method (Figure 1) can be applied to the analysis of tablets available globally.
- UV analysis of single tablets was based on to the British Pharmacopeia 2017 methodology and used as a reference method.
- This research assessed quantitatively the level of either atenolol or metformin hydrochloride in tablets from various sources.

METHODS

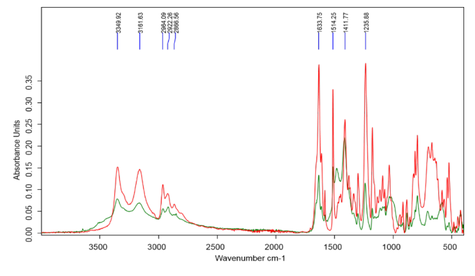
Weigh and crush test tablets

Collect sample spectra

Compare test to reference

Prepare calibration mix samples

Measure peak area



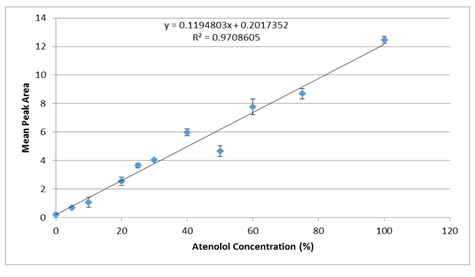


Figure 1: Preparation and analysis of test sample

Two approaches to rapid detection:

A) QUALITATIVE – simple fingerprint match under the same analytical conditions

B) QUANTITATIVE – determination of the level of API or other components

- Tablet samples are powdered using a pestle and mortar, analyses were run using a Bruker Alpha ATR FTIR system and measured over the range of 4000 – 400cm^{-1} with 2cm^{-1} resolution.
- Peak area measurements were taken at 858 – 758 cm^{-1} for atenolol and 1605 – 1501 cm^{-1} for metformin hydrochloride.

RESULTS

- The API for the tablets analysed (atenolol and metformin hydrochloride) was identified in all test samples.
- Results shown are percentage of stated dose for atenolol (Figure 2) and metformin hydrochloride (Figure 3).
- In general, the ATR FTIR (X) and UV (X) agree for the tablet batches tested.

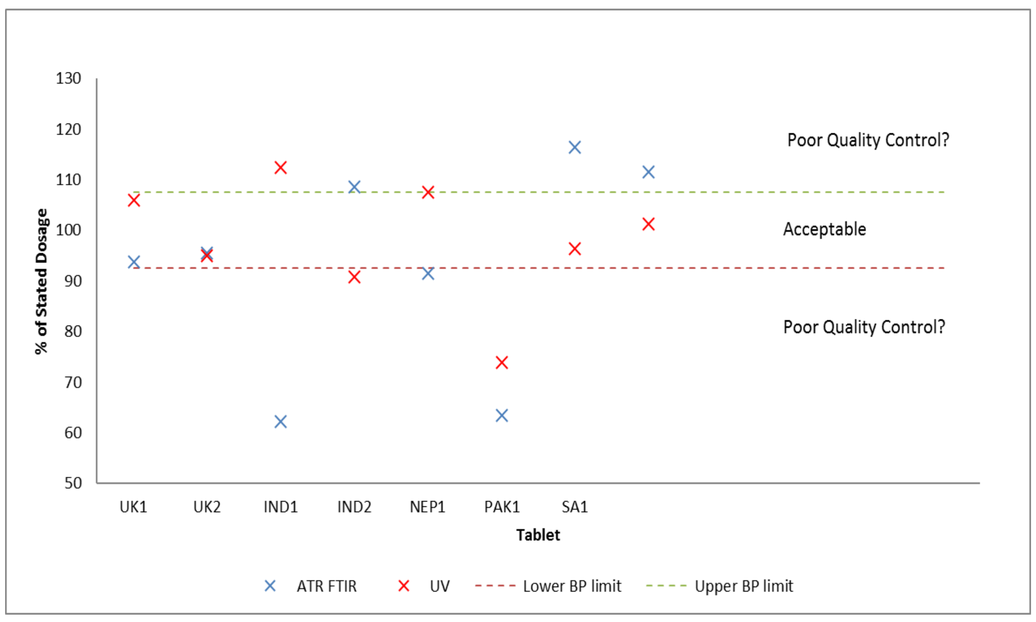


Figure 2: Percentage of stated dose of atenolol for different tablets measured by ATR FTIR and UV

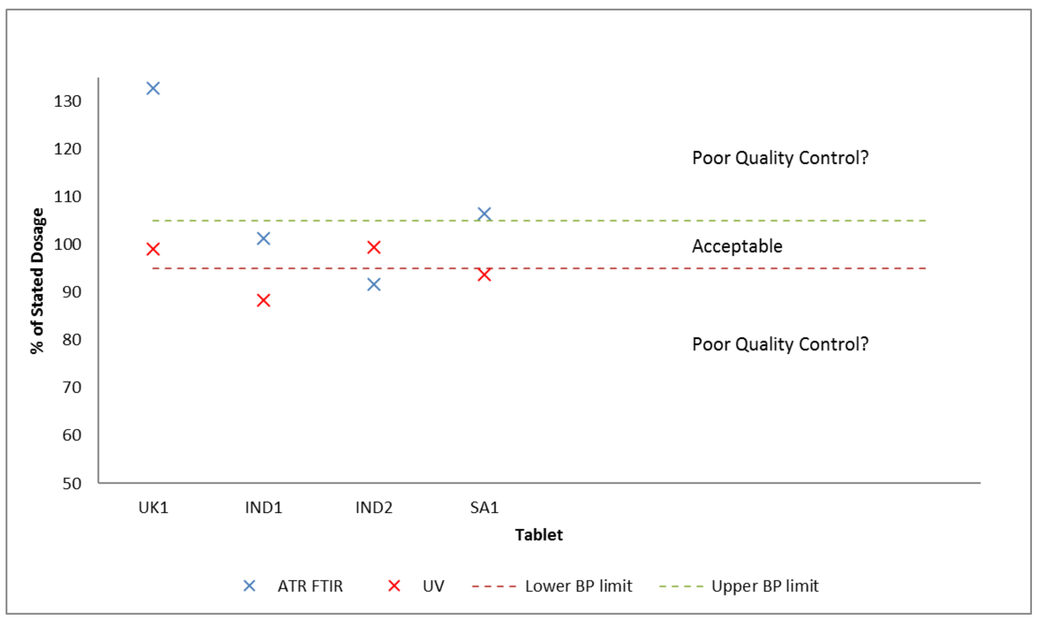


Figure 3: Percentage of stated dose of metformin for different tablets measured by ATR FTIR and UV

- Both methods identify substandard tablets, in particular the atenolol tablet PAK1 (Figure 2).
- Analysis of atenolol and metformin hydrochloride tablets was performed on individual tablets from the same batch.

DISCUSSION AND CONCLUSION

- This research demonstrates that ATR FTIR can be used as a rapid method for identifying and quantifying the target API (atenolol and metformin hydrochloride) in a crushed tablet formulation using characteristic peaks.
- Both UV and ATR FTIR analyses identified tablets exhibiting API levels indicative of poor quality control.
- This methodology reduces sampling time per tablet from an hour using conventional techniques, such as UV to < 10 minutes.
- One limitation to this technique is the requirement of ensuring the crushed sample is sufficiently mixed/ homogenised.

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#DMUengage - Helping people in Africa stay clear of fake and substandard medicines

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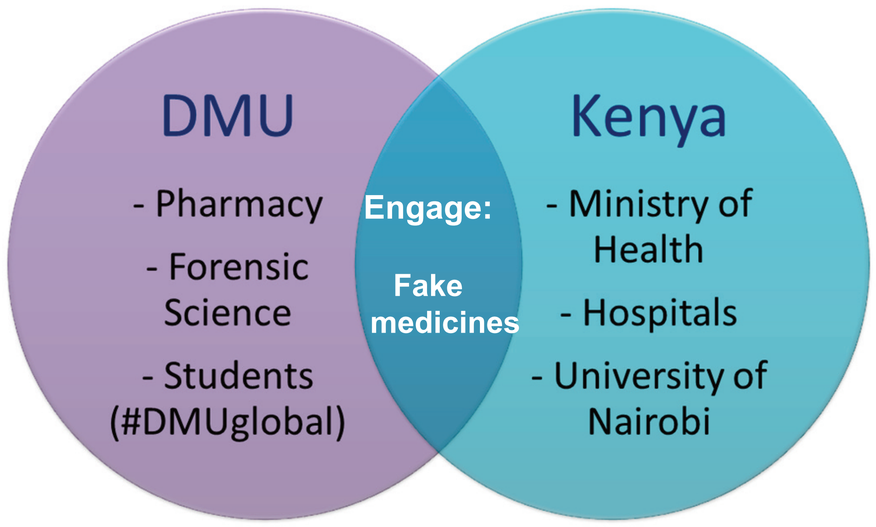
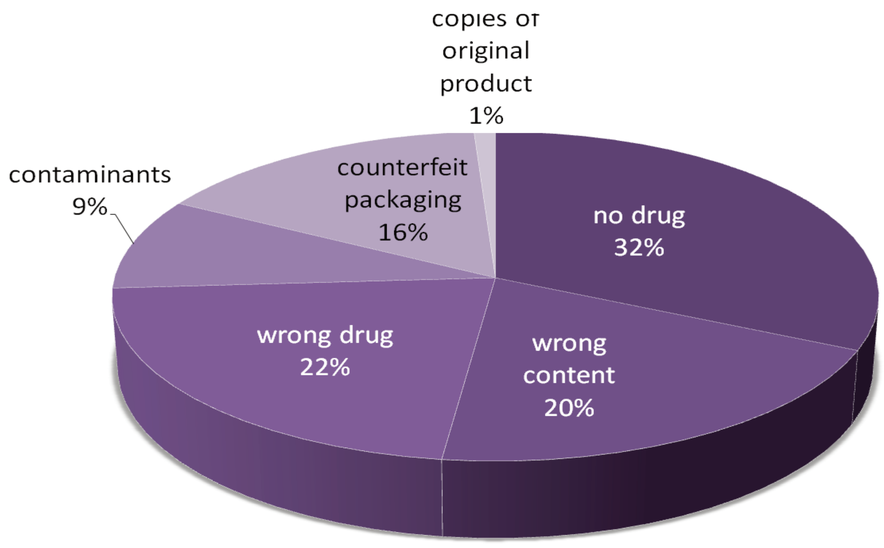
1. #DMUengage #DMUglobal visit to Kenya - June 2017



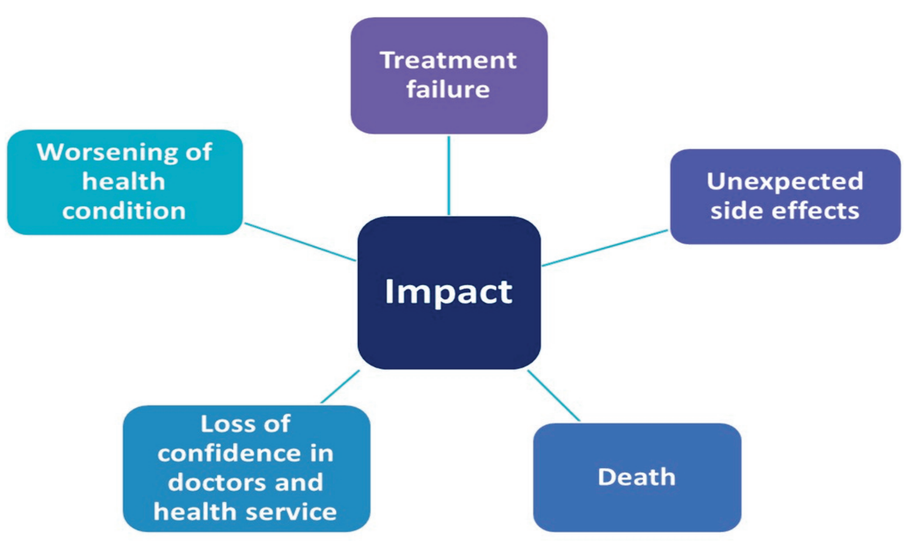
2. Counterfeit medicines - Introduction

- The growing menace of fake or substandard medicines presents a serious and increasing threat to patient safety and public health globally.
- Counterfeit medicines are a multibillion pound business, that increasingly threatens the effective delivery of health care services.
- The WHO reports that ~10% of medicines worldwide are counterfeits and this figure rises to ~30% in countries in Africa and ~50% for medicines purchased via the internet.
- Both generic and branded medicines are targeted by counterfeiters.
- In Africa counterfeit medicines have flourished due to the emergence and resurgence of many infectious diseases, particularly the three major killers: malaria, HIV/AIDS and tuberculosis.
- According to the WHO more than 120,000 people a year die in Africa as a result of fake or substandard anti-malarial drugs alone.
- Thus there is a need for a simple rapid screening process to identify suspect medicines, especially in a tablet dosage form.

3. Types of counterfeit medicines



4. Impact on patients of counterfeit medicines



5. How is a counterfeit medicine identified?

Wholesalers	• Documentation • Spot checks
Patient	• Suspect dosage form / packaging • Health not improving • Feeling unwell
Pharmacist	• Suspect packaging • Documentation • Patient reports problems
Clinician	• Patient initiated action

6. Methods for the identification of counterfeit medicines

No work up
- Visual appearence*
- Raman spectroscopy*
- Direct analysis in real time mass spectrometry

Crush
- Near infrared spectroscopy
- Infrared spectroscopy - attenuated total reflectance (ATR-FTIR)*
- Direct insertion probe mass spectrometry*
- Atmospheric solids analysis probe mass spectrometry*

Extract
- Thin layer chromatography
- Liquid / gas chromatography mass spectrometry*
- Infrared spectroscopy - standard British Pharmacopoeia method*
- Ultraviolet / visible spectroscopy*

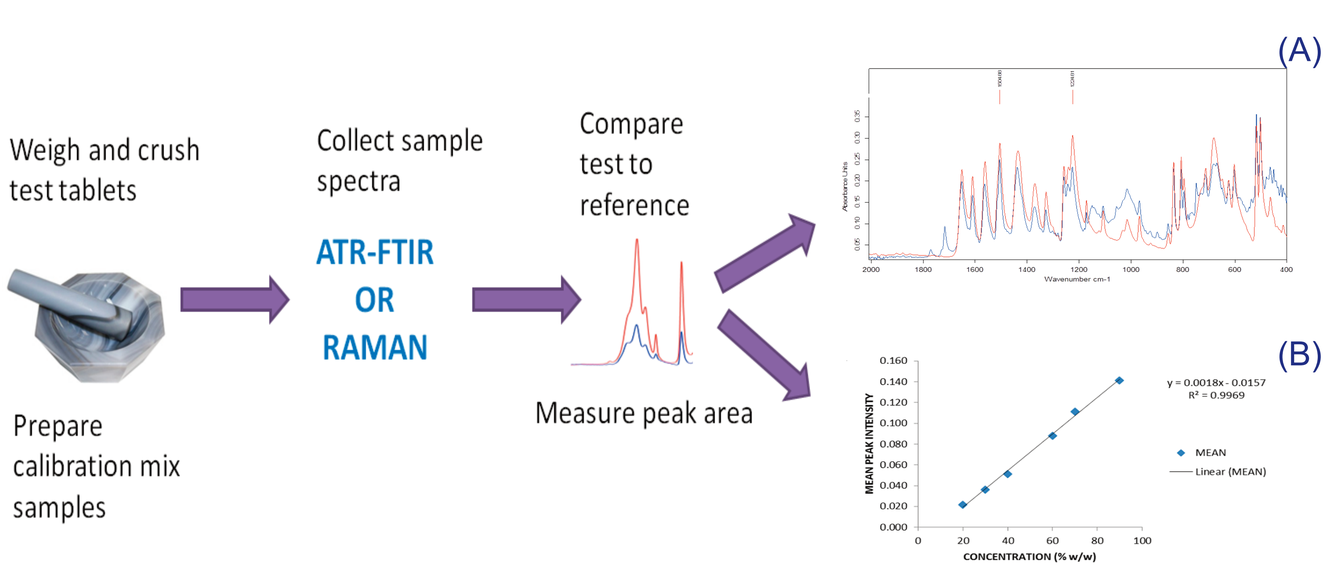
7. DMU research on the rapid detection of counterfeit medicines

- DMU carries out research in all areas shown* in 6 above.
- ATR-FTIR / Raman are investigated for rapid medication investigation.
- Mass spectrometry based techniques are used for confirmation and therapeutic drug monitoring investigations.

DMU has two approaches to rapid detection:

(A) QUALITATIVE ANALYSIS
- Simple qualitative fingerprick match under similar analytical conditions.

(B) QUANTITATIVE ANALYSIS
- Quantitative determination of the level of active pharmaceutical ingredient (drug) or other components.



8. Outcomes to date

- Exchange of information on counterfeit/substandard medicines between DMU and the following Kenya Ministry of Health departments:
  - Pharmacy and Poisons Board
  - National Quality Control Laboratory
- Student experience with Kenyan hospital pharmacy departments at:
  - Aga Khan University Hospital
  - Kenyatta National Hospital
- Integration of Pharmacy and Forensic Science students on a public health issue

9. Acknowledgements

- #DMUengage award to Dr S. Tanna
- #DMUglobal for funding student bursaries and Dr S. Tanna
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- Dr Richard Njoroge - Chief Pathologist at the Kenya National Public Health Laboratory
- Dr Shahin Sayed at the Aga Khan University Hospital, Nairobi, Kenya

Fast identification of counterfeit medicines - a comparison of two MS methods

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[2]Advion Ltd, Nottingham, UK

THE PROBLEMS

- Counterfeit medicines are becoming a major global healthcare problem.
- In developed countries counterfeits constitute 1-2% of all medicines and ~50% from the internet.
- In sub-Saharan Africa 30-40% of all medicines are thought to be counterfeit.
- Thus there is a need for a simple rapid screening process to identify suspect medicines, especially in tablet form.
- Tablets are a complex mixture of the active pharmaceutical ingredient/s (API/s) and many other materials, the excipients. The API/s constitute 1-90% of the tablet w/w depending on the dose level.
- For screening purposes there is the need to confirm the presence of the API/s with minimal sample work-up and specifically no solvent extraction in order to increase throughput.

METHODOLOGY

- This research compares the direct mass spectrometer (MS) analysis of ions produced from crushed tablet samples introduced into the analyser by two different sampling probe techniques.
- The two systems are shown schematically in Figure 1(a) and (b).

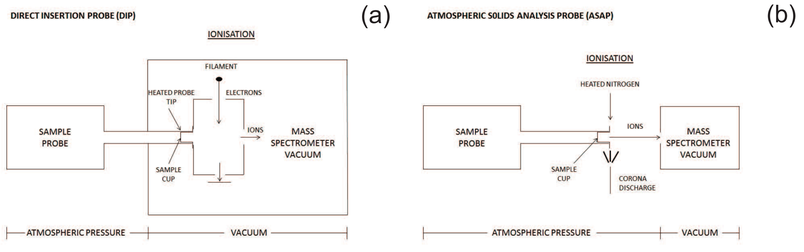

Figure 1. Schematic representaion of Direct Insertion Probe (DIP) (a) and Atmospheric Solids Analysis Probe (ASAP) (b)

- Both systems have a demountabale probe with replaceable cups in the tip to hold powder or liquid samples.
- Both systems have a simple quadrupole MS with unit mass resolution scanning over the *m/z* range 50-500.
- The differences between the ease of use and the results from the systems are derived from the means of sample volatilisation and subsequent production of positive ions.

Direct Insertion Probe (DIP)

- Bruker 300-MS, Bruker Instruments, UK.
- Once the sample is placed in the tip the probe has to be inserted into the MS vacuum system via a vacuum lock.
- The probe tip was electrically heated over the range 40-300˚C.
- Volatilised material was ionised by 20eV electron impact.
- Ions were focussed into the Bruker 300-MS for analysis.

METHODOLOGY

Atmospheric Solids Analysis Probe (ASAP)

- Advion Compact MS (CMS), Advion Ltd, UK.
- Once the sample is placed in the tip the probe is inserted directly into the ionisation region and the flow of heated nitrogen volatilises the API/s.
- Ionisation occurs by protonation in the corona discharge field.
- Ions are focussed into the Advion Compact MS for analysis.

Materials

- Reference samples of paracetamol, caffeine, atenolol, chloroquine phosphate, sulfadoxine and the excipients magnesium stearate and lactose were obtained from Sigma Aldrich, UK.
- Literature data was used for the identification of the MS data from artmether and lumifantrine tablets.
- Tablet samples were obtained from various ‘pharmacy’ sources in India, Nigeria, Nepal, China and Rwanda and compared with samples from the UK and Europe and with the reference data.

RESULTS

- Electron impact ionisation in the DIP process leads to molecular fragmentation with a consequent reduction in the intensity of the molecular ion.
- The fragmentation pattern is a characteristic fingerprint of that compound which can be used for identification.
- Proton transfer, in the ASAP system, is a soft ionisation process which should enhance the presence of the pseudo-molecular ion at $M+H^+$ and thereby produce a much simpler spectrum suitable for screening purposes.

Reference sample data

- Samples of the pure reference materials were analysed by both systems.

API	RMM (MWt)	*m/z* DIP	*m/z* ASAP
Paracetamol	151	151, 109	152, 110
Caffeine	194	194	195
Chloroquine phosphate	516	319, 86	320, 247
Atenolol	266	222, 223	267,190, 225
Sulphadoxine	310	246, 245, 227	311, 156

- Tablet samples containing these API/s either singly or in combinations, were collected and submitted to both analytical methods.

Single API in a tablet

- Paracetamol samples consistent with the expected reference data.

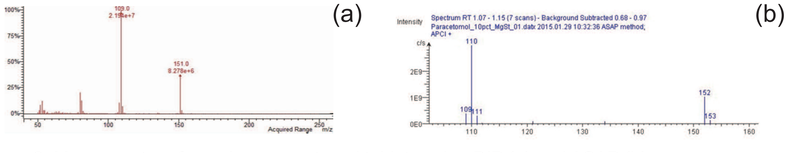

Figure 2. Mass spectra of genuine paracetamol tablets using DIP (a) and ASAP (b)

RESULTS

- Paracetamol samples from China and India giving inconsistent spectra.

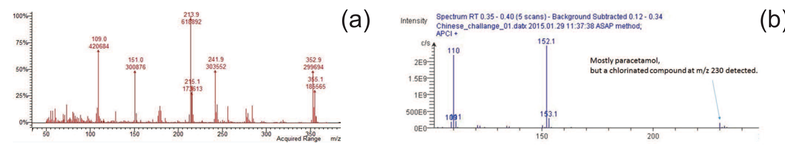

Figure 3. Mass spectra of suspect paracetamol tablets using DIP (a) and ASAP (b)

Two APIs in a tablet

- Both APIs in a single tablet can be identified.

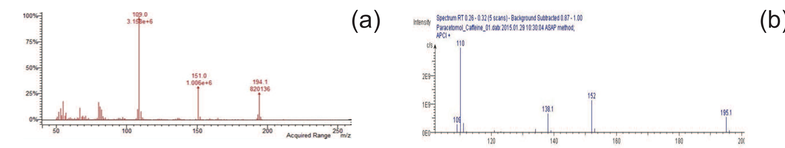

Figure 4. Mass spectra of tablets containing paracetamol and caffeine using DIP (a) and ASAP (b)

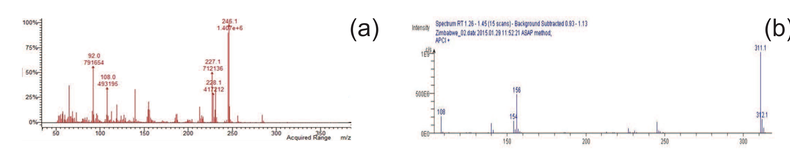

Figure 5. Mass spectra of sulphadoxine and pyrimethamine tablets using DIP (a) and ASAP (b)

DISCUSSION

- The ASAP mass spectra were very simple even from complex tablet formulations. The presence of single and multiple APIs could be confirmed.
- The ASAP probe analysis of antimalarial tablets gave for chloroquine phosphate *m/z* 320, 247 (RMM 516). Multicomponent tablets were more challenging; lumifantrine gave *m/z* 530, 528 (RMM 529) and artemether produced fragment ions at *m/z* 221 (RMM 298). Artensuate produced ions at *m/z* 321, 325 (RMM 384) and amodiaquine at *m/z* 357 (RMM 356).
- The DIP probe relied on the more energetic electron impact ionisation which produced some fragmentation of the parent molecule. In several cases this resulted in less informative spectra. The EI observed signals were: paracetamol *m/z* 151, 109; caffeine 194, 109; chloroquine phosphate *m/z* 319, 86.
- The multicomponent antimalaria tablets were difficult; lumifantrine and artemether tablets produced a single fragment ion at *m/z* 142. Artensuate produced ions at *m/z* 69, 81 (RMM 384) and amodiaquine at *m/z* 356, 357 (RMM 356).

CONCLUSIONS

- Overall the ASAP probe system produced data more rapidly and with less potential for contamination of the ionisation region. The ASAP mass spectra were generally simpler which was ideal for use as a rapid screening methodology to identify counterfeits in a QC scenario.

Identification of counterfeit pills - Is rapid instrumental analysis possible?

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BACKGROUND

- From 'acceptable' counterfeits of designer items to fake anti-cancer medicines counterfeiting is a growing worldwide criminal trend that affects us all.
- The WHO estimate that ca. 10% of medicines sold worldwide are counterfeit[1] whilst Pfizer estimates that 80% of all internet sales of Viagra are counterfeit[2].
- More than 400 patients in Pakistan died as a result of poor quality heart disease medicines and untold hundreds of Africans following treatment with substandard anti-malarial medication.
- The authentification of in-dose medication forms usually relies on secure packaging and tracking codes in combination with human senses. This approach is necessary to maintain the volume of medicines imported into a country. Fake packaging never hurt a patient.
- In reality it is the dosage form that must be acceptable and therefore a rapid test taking only a few minutes to establish the quality of the dosage form would be an extremely valuable tool.

INTRODUCTION

- Some of the options for investigating a suspect counterfeit medicine are shown in Fig. 1.

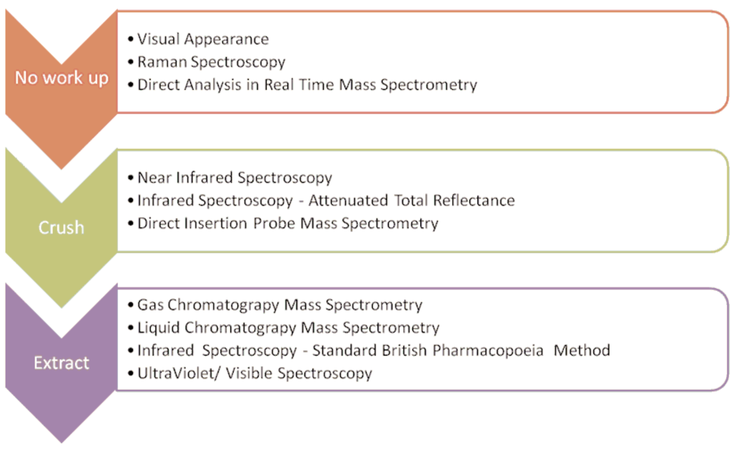

Figure 1. Options for investigating counterfeit medicines

- The time taken for each level increases from minutes for the top 2 to many hours for the in depth analyses.
- Infrared (IR) techniques offer a convenient method for the identification of pharmaceutical active ingredients (AI).
- The conventional approach uses solvent extraction followed by KBr disc preparation and IR transmission analysis. Whilst this approach is designed to remove the AI from the excipients it is lengthy and has several drawbacks:
  - KBr is hygroscopic and not easy to handle or store
  - Difficult to make good KBr disc and need a hydraulic press
  - Two much sample in the disc results in poor spectra
- These disadvantages can all be overcome by the use of the Attenuated Total Reflection (ATR) IR mode. The basic principle is shown in Fig. 2.

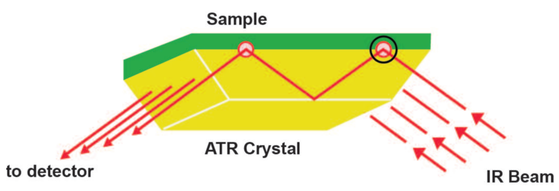

Figure 2. ATR principle

- The sample is placed on the top of the ATR crystal and the incident beam is attenuated at the sample/crystal interface before detection.
- Any sample that can maintain an intimate contact with the crystal can therefore be examined.
- The crucial questions in terms of the proposed application are:
  - Will the ATR spectrum match the reference spectrum for the AI selected?
  - Can a fingerprint IR spectrum be obtained in the presence of excipients?
  - Can therapeutic levels of AIs be identified in tablet/liquid formulations?
- A range of tablets were used to examine the potential of the ATR technique.

METHODS

- All of the analyses were run using the Bruker Alpha ATR FTIR system scanning over the range 2000 - 600 cm^{-1} with 4 cm^{-1} resolution.
- Test materials were over-the-counter formulations or prescription dosage forms from the NHS in the UK. These were tablets containing Aspirin, Caffeine, Paracetamol, Ibuprofen, Atenolol and Viagra.
- The general experimental approach was as follows:
  - The test tablet was crushed and separate samples of the powder were individually analysed to test the reproducibility versus granule size.
  - The resultant spectra were compared with reference data.
  - The detection limit for the AIs was investigated by preparing known concentrations of the AI in a pre-prepared excipient mixture[3].

RESULTS

- Examples of the agreement of the ATR spectra with reference spectra are shown in Fig. 3.

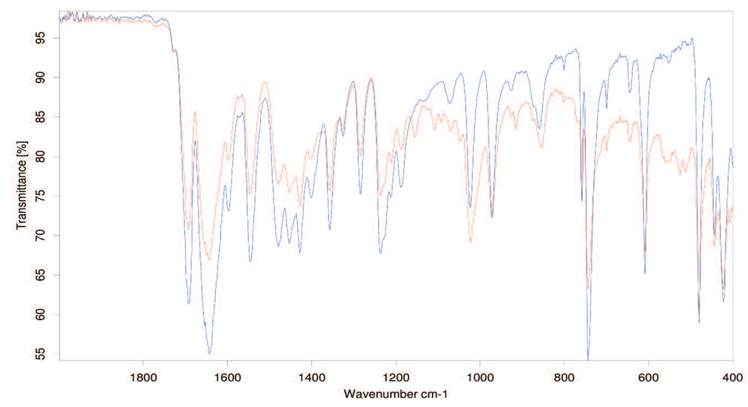

Figure 3. A comparison of the ATR spectrum for a caffeine tablet (red) and caffeine standard (blue)

- The results for the fingerprint determinations and the LODs of the AIs in excipient mixtures are detailed in Table 1.

Table 1. Summary of quantitative data

Pharmaceutical Active Ingredient (AI)	ID at Dose Level	ID at 5% w/w
Aspirin	Yes	Yes
Caffeine	Yes	Yes
Paracetamol	Yes	Yes
Ibuprofen	Yes	Yes
Atenolol	Yes	-
Viagra	Yes	-

- Pain relief tablets often contain several AIs. Such tablets were studied to see if peaks characteristic of the stated components could be identified (Table 2).

Table 2. Summary of qualitative data for pain relief tablets

Pharmaceutical Active Ingredients (AIs)	Result
Aspirin + Caffeine	Characteristic peaks observed for both
Aspirin + Caffeine + Paracetamol	Characteristic peaks observed for all three

- Fig. 4 shows the spectrum from a Viagra reference sample. Comparsion of regions of the spectrum obtained from an 'Internet Viagra' sample with the reference (Fig. 5) showed sufficient differences to raise questions of authenticity.

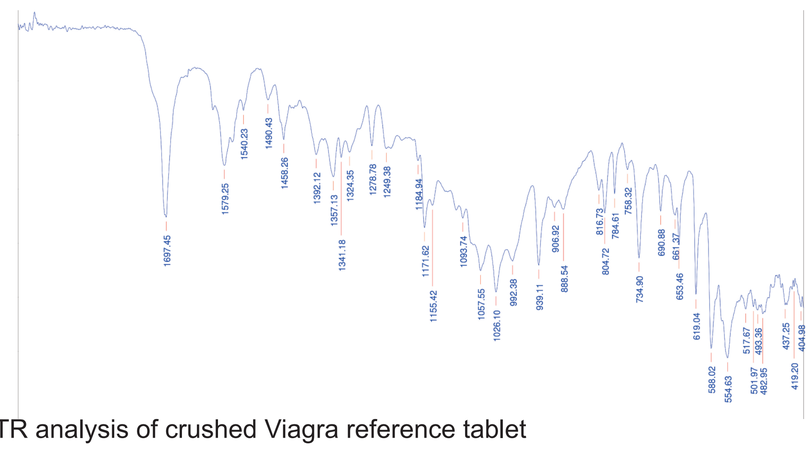

Figure 4. Direct ATR analysis of crushed Viagra reference tablet

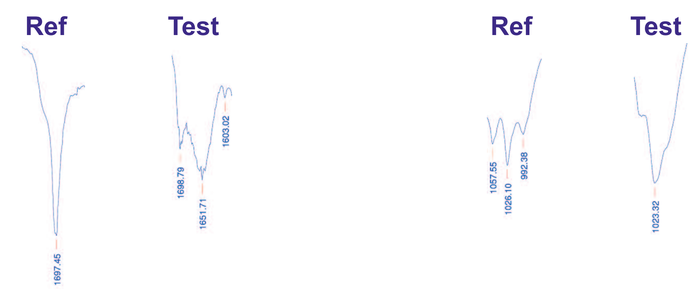

Figure 5. Comparison of regions of the ATR spectra for reference and test Viagra tablets

DISCUSSION AND CONCLUSION

- The simple ATR approach provides a means of rapid assessment of the medication in the presence of the excipients.
- Within the limits of the present study the presence of stated AIs can be confirmed.
- The ATR system is able to identify material for further investigation rapidly based on dry powder analysis.

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ACKNOWLEDGEMENTS

- Bruker UK

A Microscopic Approach to a Big Problem...

...Analysing Counterfeit Drugs

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BACKGROUND

- The World Health Organisation (WHO) estimates up to 1% of all medicines in developed countries are likely to be counterfeit, whilst in developing countries it is estimated that 33% of medicines are counterfeit[1].
- India is the leading supplier of low cost generic drugs to Africa[2], and it has been reported that India leads all countries in producing and exporting counterfeit medicines[3].
- As much as 35% of worldwide counterfeit sales comes from India, with Pakistan accounting for 13.3% of sales[3].
- Counterfeit medicines have been known to use insufficient amounts of active ingredient in their products to enable them to pass qualitative screening tests. As a result, patients receive lower than expected doses of the drug, leading to ineffective treatment, therapeutic failure and even drug resistance.
- Counterfeiters are already finding ways to bypass established techniques, therefore the development of new techniques is required.

AIMS AND OBJECTIVES

- The overall aim of this project is to develop a novel method for the rapid identification of counterfeit drugs.
- To achieve this, spectroscopic methods including Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) will be applied to dosage forms and compared to suitable references.

RESULTS

- Samples of Atenolol (used for the treatment of high blood pressure) were obtained from Pakistan and India and were compared to UK manufactured samples.
- Cross sections of the Atenolol tablets were analysed using SEM/EDX to investigate if the spatial array of selected elements could provide a novel technique to fingerprint tablets.

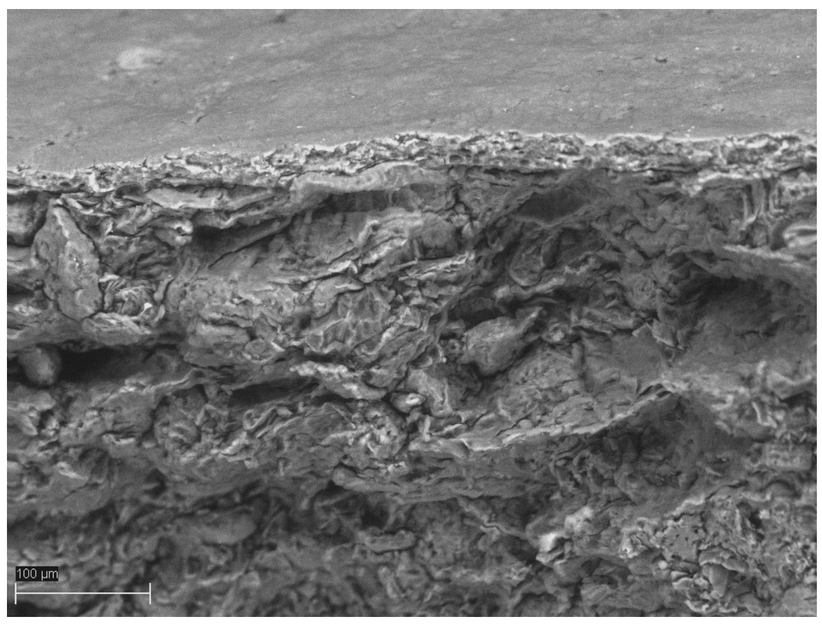

Figure 1a - Backscatter image of UK sample

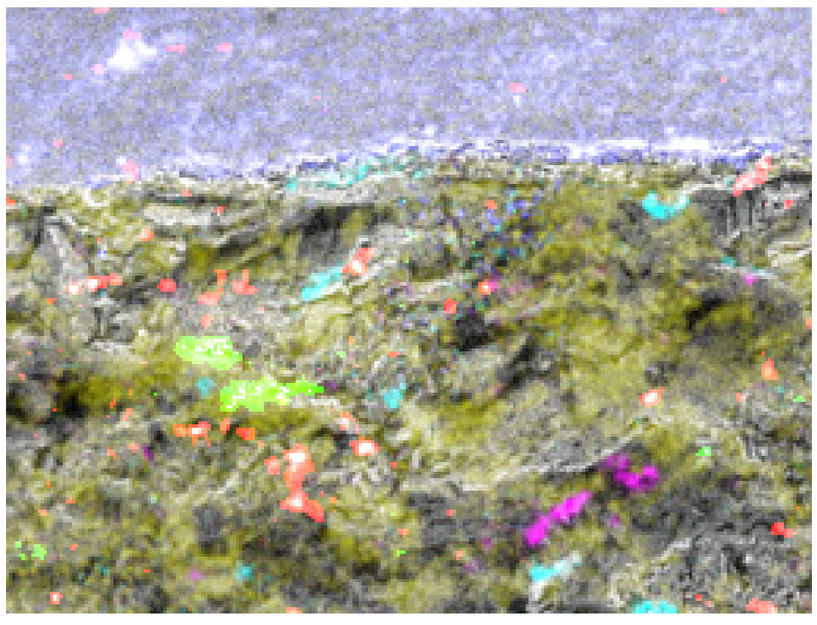

Figure 1b - EDX map of UK sample

Figure 2a - Backscatter image of Indian sample

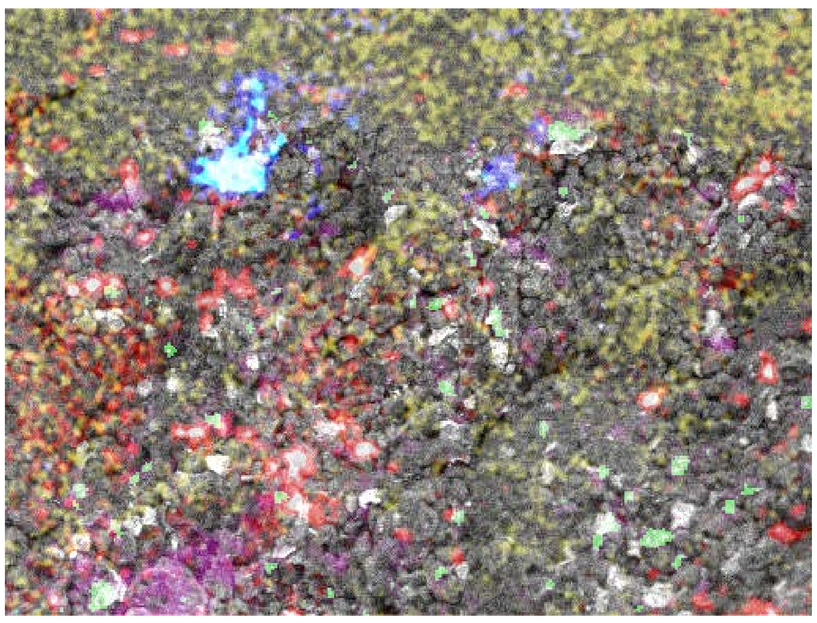

Figure 2b - EDX map of Indian sample

CONCLUSION AND FURTHER WORK

- Backscatter images of the UK and Indian sample show noticeable compositional and topographical differences.
- EDX analysis confirms the compositional differences, in particular the absence of Titanium and Aluminium and presence of Phosphorus in the Indian sample.
- Promising results have already indicated that other detectors on the SEM in conjunction with this work may be suitable to detect counterfeit medicines, further work is needed to develop and validate this novel method.
- Current published methods focus on a specific formulation, however this method has the potential for universal formulations.

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It’s not what it says on the packet...
...Counterfeit drugs

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INTRODUCTION

- The World Health Organisation (WHO) estimates up to 1% of all medicines in developed countries are likely to be counterfeit, whilst in developing countries it is estimated that 33% of medicines are counterfeit[1].
- The figure for internet sales, may be as high as 50% from websites that conceal their physical address[1& 2]. It is believed 2.5 million men in Europe haven taken counterfeit Viagra, purchased online[3].
- The United Nations (UN) estimates worldwide trade in counterfeit medicines is worth $500 billion with West Africa one of the key destinations[4].
- Usage of counterfeit medicines can result in treatment failure, drug resistance or even death. The International Policy Network blames counterfeit medicines for 700,000 deaths worldwide from Malaria and Tuberculosis[4].
- Forensic investigation of counterfeit medicines can be time consuming, costly and usually destructive.

AIMS AND OBJECTIVES

- The overall aim is to develop a novel method for the rapid identification of counterfeit drugs.
- To achieve this spectroscopic methods will be applied to packaging and dosage forms and compared to suitable references.
- Methods which eliminate the need for extensive sample preparation will also be developed.

PRELIMINARY RESULTS

- Suspect samples of Atenolol were obtained from Pakistan and compared to genuine samples.
- Samples were visually analysed (Figure 1 a and b) and tested to BP standards using UV/Vis (Figure 2).
- Cross sections of Ibuprofen tablets were analysed using SEM/EDX (Figure 3) to see if the spatial array of selected elements can provide a mechanism to fingerprint a tablet.

Figure 1a – Visual Appearance (Packaging)

Genuine Sample

Suspect Sample

The external packaging of genuine and suspect Tenormin branded Atenolol were visually compared. Results show that the suspect sample carries ICI’s logo in addition to AstraZeneca’s.

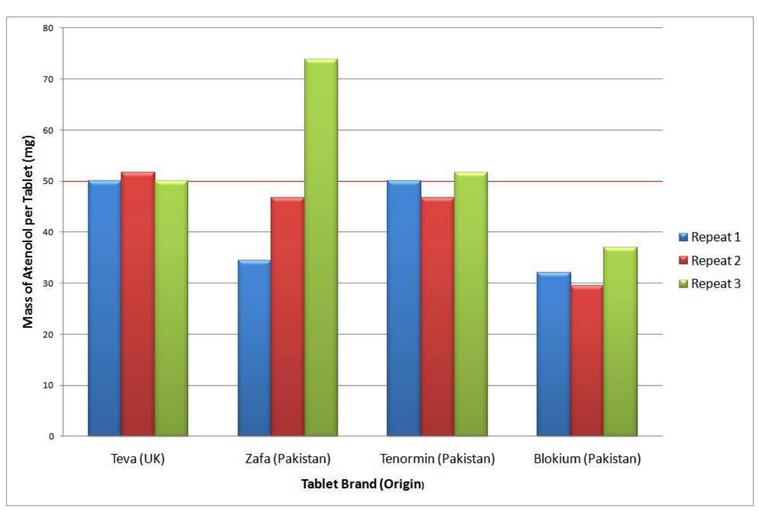
Figure 1b – Visual Appearance (Tablet)

Genuine Sample

Suspect Sample

Genuine and suspect Tenormin tablets were visually compared. Analysis of the images shows significant differences, the genuine sample has the brand name on one side. The ‘105’ on the reverse side is the manufacturer’s code for a 50mg tablet. The suspect sample shows the brand name and dose level on one side and is blank on the reverse. In addition the score mark is missing from the suspect sample.

Figure 2 – UV/Vis Analysis of 50mg Atenolol Tablets



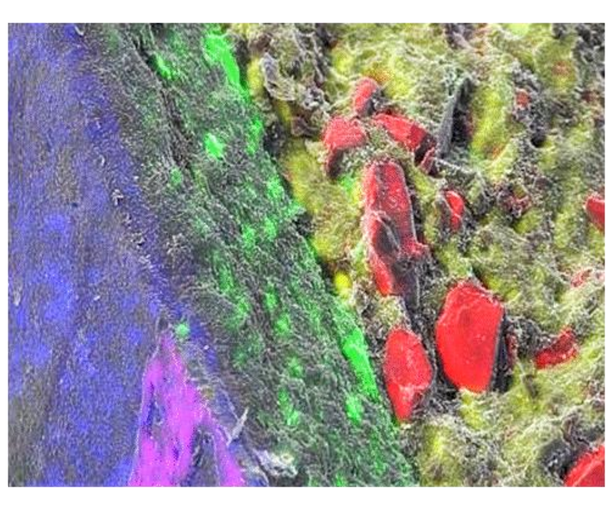
3 sets of Atenolol samples were acquired from Pakistan and dose levels were compared to a genuine sample from the UK (Teva). Results indicate that the Blokium tablets tested were sub-therapeutic. Zafa tablets exhibited poor uniformity, possibly as a result of inadequate mixing. Tenormin tablets and Teva tablets are comparable.

Figure 3 – SEM/EDX Analysis of Ibuprofen

Results show the distribution of elements within the cross section of the tablet.

- Titanium
- Iron
- Magnesium and Silicon
- Sodium
- Carbon

The presence of different elements can indicate certain excipients.



CONCLUSIONS

- Visual inspection shows strong indications that one sample is counterfeit.
- Preliminary UV/Vis results show that Blokium tablets tested contained sub-therapeutic dose levels.
- UV/Vis results show that Zafa tablets tested did not contain uniform content of Active Pharmaceutical Ingredient (API).
- SEM/EDX has potential for identifying different tablet types and further work is aimed at differentiation of tablets of similar generic formulations.

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